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CONTENTS

THE OXIDATION OF SULFUR IN ALKALI SOIL AND ITS EFFECT ON THE REPLACEABLE BASES*

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HISTORICAL INTRODUCTION

Numerous investigators have observed that many of the unfavor-able physical and chemical properties of alkali soils are caused by a displacement of the normal soil bases. This displacement is brought about by the predominant bases of the soluble salts in the soil. In the treatment of alkali soils it is important, therefore, to bring about a reversal of this process to the end that the normal relationship of the replaceable bases may be ultimately restored. Various materials have been used for this purpose. Sulfur is of interest in this con-nection, since by its oxidation the necessary chemical changes may be brought about.

There are two sets of factors to be considered in the use of sulfur on alkali soils. First, the conditions influencing the oxidation, such as the effect of the soluble salts, varying alkaline reaction, aeration, etc. Second, the effect of the oxidation product, sulfuric acid, upon the soil. Previ us studies on the oxidation of sulfur in alkali soils have been very lim ed and little is known concerning the influence of the concentratio sodium salts, alkalinity, etc., upon the speed of the reaction.

Sulfur is mainly oxidized by biological action and the process is extracellular and autotrophic. *Thiobacillus thioparus* studied by Nathansohn,[21] Beijerinck, [1] and Jacobsen,[12] *Thiobacillus "B"* and *Thiobacillus thio-oxidans,* studied by J. G. Lipman and his associates,*[18] oxidize sulfur very readily and these organisms may be present in alkali soils.

The non-biological oxidation of sulfur has been studied by Kappen and Quensel,[13] Brown and Kellogg,[2] and MacIntyre, Gray, and Shaw.[19] The last named work was the most extended, in connection with which it was shown that non-biological oxidation of sulfur is not of great importance.

Hibbard[11] neutralized the alkalinity of soil by applying sulfur, and Rudolfs[22] reported that as a result of sulfur oxidation a desirable change takes place in the reaction and physical properties of alkali soils.

Kelley and Thomas[16] found that "sulfur undergoes reasonably active oxidation" in soils which contain large amounts of sodium salts. They found that the amount of sulfur necessary for the neutralization of the soil was equivalent to two or three times the soluble sodium carbonate. Since the work of Cummins and Kelley[4] and others shows that the exchange complex of alkaline soils may be sodium saturated, Kelley and Thomas believe that the excess of sulfur was used in double decomposition with the sodium complex of the soil.

The exchange properties of soils were first noted by Thompson[24] and then studied in detail by Way[26] who concluded that the active replacement material is a hydrated aluminum silicate. He later prepared silicates which exhibited the same type of base exchange. Eichhorn[6] and others[7, 17] have confirmed and amplified Way's results. Van Bemmelen[25] ascribed the exchange phenomena to the extended surface of the soil particles. Gans,[8] however, showed that the analogous base exchange property of artificial zeolites is chemical and that it depends upon their composition and structure and is independent of the size of the particles.

Gedroiz[10] concluded that with the exception of ammonia the replacing activity varies directly with the atomic weight and valence of the cation, and that the exchange capacity of a soil is a definite quantity. The exchange reaction was found to be reversible, and the equilibrium to be dependent upon relative masses and the atomic weight and valence of the salt. The reaction is instantaneous and is assumed to be a surface phenomenon.

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* For a very complete review of the sulfur oxidizing organisms see "Biochemical oxidation of sulfur and its significance to agriculture," by J. S. Joffe, New Jersey Agr. Expt. Sta. Bul. 374:1–91. 1922.

The Formation of Alkaline Soils.—The change produced by soluble salts on the composition of the reactive constituents of soils is of great significance in the formation of alkaline soils. The accumulated sodium salts, chiefly NaCl and Na_2SO_4, displace the calcium which predominates in productive soils[15]*leaving sodium in its place. Upon leaching, either natural or artificial, the soil becomes alkaline. This alkalinity is considered by Gedroiz to be due to the hydrolysis of the sodium silicate complex. He concludes that calcium carbonate increases the soluble alkalinity according to the following reaction:

$$2\,NaX^* + CaCO_3 = CaX + Na_2CO_3.$$
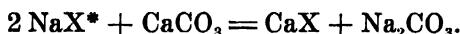
Gedroiz[9] holds that the saline accumulations are the primary cause of soil alkalinity and the above equation suggests that the formation of sodium carbonate, through the action of calcium carbonate, is a step in the reclamation of the soil.

Dominicis'[5] theory is in agreement with that of Gedroiz' except as to the necessity of calcium carbonate for the formation of sodium carbonate. He states that the hydrolysis of the sodium complex results in the formation of sodium hydroxid which reacts with carbon dioxid to form sodium carbonate. Cummins and Kelley[4] have experimentally demonstrated the presence of sodium hydroxid when carbon dioxid was excluded from the system.

Relation between the Physical Characteristics and the Replaceable Bases.—The modification of a soil with a high content of replaceable calcium to a soil low in calcium and high in replaceable sodium results in profound changes in the physical properties of the soil. These changes were noted by Gans[8] in artificial zeolites and in soils receiving large applications of sodium nitrate. He also mentions the presence of a brown to black surface crust in these soils.

Sharp[23] has shown the increased colloidality produced in soils by treatment with salts (NaCl, Na_2SO_4, Na_2CO_3, and NaOH) by drying and weighing the material which remained in suspension in water. He clearly points out the relationship of the sodium replacement to the resulting deflocculated condition, which he ascribed to the sodium complexes thus formed.

A satisfactory chemical explanation of the potency of the divalent bases as flocculants and of the monovalent bases as deflocculants is not available. Among the more recent papers are those of Comber and Mattson. Mattson[20] ascribes the flocculating action of calcium hydroxid to the anomalous adsorption of negatively charged hydroxyl-

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* This symbol is used throughout the paper to indicate the complex involved in replacement.

ions upon particles which are already negatively charged. This increase in the negative charges attracts the bivalent cation, calcium, which forms binding links and thus flocculates the particles. The monovalent ions possess no such linkage capacity.

Comber[3] differentiates between the colloidal nature of the core and the emulsoid surface of a clay particle. Calcium hydroxid is absorbed from dilute solutions only by the core of the particle and this process produces deflocculation. Absorption of calcium hydroxid in greater amounts results in its distribution on the emulsoid surface with the resulting flocculation of the particles.

EXPERIMENTAL METHODS AND RESULTS

Four different soils were used in these studies. Soil 5187 is an alluvial sand from the river-bottom lands west of Riverside, California. It is slightly alkaline and high in total soluble salts, and contains an abundance of calcium carbonate. Soil 5188 is a saline soil but not alkaline in reaction and contains practically no calcium carbonate. It is a fine sandy loam of the Fresno series and was obtained from an olive orchard on the Kearney Ranch, Fresno, California. Soil 5189 contains large amounts of sodium carbonate and is also a fine sandy loam of the Fresno series, similar in origin to soil 5188. The sample was taken from the worst portion of the University of California's Experimental Reclamation Tract on the Kearney Ranch, Fresno, California. It contains only small amounts of calcium carbonate. Soil 5190 is high in both soluble salts and calcium carbonate. It is a fine sandy loam of the Jordan series and was taken from the Terminal Sub Station Experimental Tract at Salt Lake City, Utah.*

Each of these samples was air dried, thoroughly mixed by screening and stored in large wooden bins. With the exception of soil 5188, which shows only slight effects of the salts upon olive trees growing in it, the areas from which the samples were taken were barren.

Sulfofication Experiments.—A set of sulfofication experiments was set up, using loosely covered two-quart Mason jars as containers. Sulfur was mixed with the soils in amounts varying from 0.15 to 2.00 per cent. The soils were kept near the optimum moisture content and samples were withdrawn for analysis at bi-weekly intervals.

* This sample was obtained through the kindness of Mr. R. A. Hart, Senior Drainage Engineer, United States Department of Agriculture, Salt Lake City, Utah.

The results expressed as parts per million of dry soil are reported in tables 1 and 2. These experiments, while primarily serving as an indication of the rate and extent of sulfur oxidation in these soils, have afforded further confirmation of the conclusions previously reached in this and other laboratories: namely, that sulfur readily undergoes oxidation in very alkaline soils and that this oxidation may ultimately result in the production of a neutral or acid reaction in the soil. The results from soils 5188 and 5189 offer an interesting contrast in that sulfofication was the more rapid in the soil which contained considerable sodium carbonate. It is also noteworthy that the alkaline soil contained much the higher total concentration of soluble salts.

TABLE 1

SULFOFICATION EXPERIMENTS WITH VARYING AMOUNTS OF SULFUR

Weeks	0.15 per cent S				0.25 per cent S				0.50 per cent S				1.00 per cent S			
	CO_3	HCO_3	SO_4	pH	CO_3	HCO_3	SO_4	pH	CO_3	HCO_3	SO_4	pH	CO_3	HCO_3	SO_4	pH
SOIL 5189																
0	1170	854	4354	9.8+	1170	854	4354	9.8+	1170	854	4354	9.8+	1170	854	4354	9.8+
2	1060	1170	4587	9.8+	1030	1090	4798	9.8+	1080	1070	4756	9.8+	1000	1130	4819	9.8+
4	980	810	4891	9.8+	960	910	5012	9.8+	880	960	5121	9.8+	890	1000	5364	9.8+
6	900	740	5186	9.8+	820	780	5301	9.8+	760	740	5505	9.8+	700	700	5739	9.8+
8	830	940	5414	9.8+	740	860	5779	9.8+	710	820	5919	9.8+	650	880	6204	9.8+
10	760	1010	5681	9.8+	710	1100	6143	9.8+	620	980	6387	9.8+	510	910	6616	9.8+
12	720	1230	5814	9.8+	680	980	6527	9.8+	550	1020	6843	9.8+	430	870	7120	9.8+
14	640	950	6020	9.8+	660	1070	6817	9.8+	470	1110	7184	9.8+	390	1080	7597	9.8+
16	510	810	6182	9.8+	530	1210	7084	9.8+	410	1190	7519	9.8+	360	1220	8064	9.8+
18	440	830	6307	9.8+	410	1140	7309	9.8+	430	1040	7921	9.8+	220	1160	8413	9.8+
20	320	1210	6451	9.8+	280	1320	7557	9.8+	190	1410	8347	9.4	111	1280	9012	9.0
22	270	930	6693	9.8+	160	1670	7721	9.6	104	1360	8562	9.4	58	1210	9306	8.6
24	260	1540	6814	9.8+	130	1980	7936	9.4	86	1310	8877	9.2	44	1130	9541	8.6
SOIL 5190																
0	211	409	3622	9.2	211	409	3622	9.2	211	409	3622	9.2	211	409	3622	9.2
2	150	370	4118	8.8	154	380	4148	8.8	140	342	4207	8.8	160	210	4251	8.8
4	136	312	4354	8.8	130	306	4519	8.8	108	288	4487	8.8	100	164	4560	8.8
6	110	288	4661	8.8	102	254	4711	8.6	90	210	4880	8.6	58	236	4767	8.6
8	64	240	5001	8.6	50	272	5107	8.4	42	254	5088	8.4	18	202	5212	8.4
10	22	270	5239	8.2	18	220	5340	8.2	10	276	5418	8.2	0	198	5671	8.2
12	10	224	5427	8.2	8	234	5561	8.2	0	214	5819	8.0	0	516	6033	7.8
14	0	306	5711	8.0	0	212	5841	8.0	0	480	6107	7.8	0	764	6354	7.8
16	0	418	5821	8.0	0	368	6012	7.8	0	615	6315	7.8	0	914	6580	7.8
18	0	560	6020	7.8	0	548	6241	7.8	0	976	6531	7.8	0	1130	6760	7.6
20	0	720	6345	7.8	0	872	6416	7.8	0	850	6844	7.6	0	1462	7003	7.4

TABLE 2

SULFOFICATION EXPERIMENTS WITH SOILS CONTAINING TWO PER CENT OF SULFUR

Soil 5189							Soil 5188					
Weeks	SO₄	CO₃	HCO₃	Ca	Mg	pH	Weeks	SO₄	CO₃	HCO₃	Ca	Mg	pH

Weeks	SO$_4$	CO$_3$	HCO$_3$	Ca	Mg	pH	Weeks	SO$_4$	CO$_3$	HCO$_3$	Ca	Mg	pH
0	4354	1170	854	0	0	9.8+	0	1437	0	156	68	18	7.4
2	4601	950	1060	0	0	9.8	2	1671	0	305	134	31	7.4
4	5004	980	810	0	0	9.8	4	1901	0	196	226	40	7.4
6	5224	1120	870	0	0	9.8	6	2354	0	88	402	64	7.2
8	5571	780	1210	0	0	9.8	8	2418	0	34	467	60	7.2
10	5824	430	1450	0	0	9.8	10	2461	0	40	480	72	7.2
12	6212	370	1780	0	0	9.8	12	2512	0	54	482	63	7.2
14	6784	230	1990	0	0	9.8	14	2498	0	28	511	68	7.0
16	7627	140	2200	0	0	9.8	16	2524	0	20	501	51	6.8
18	8523	72	1410	0	0	9.2	18	2560	0	32	520	72	6.8
20	9419	32	1090*	18	0	8 4	20	2540	0	18	524	68	6.8
22	10287	0	1010*	309	41	8.0	22	2585	0	18	555	70	6.8
24	10940	0	940*	518	68	7.6	24	2611	0	24	573	72	6 8
26	11817	0	809*	842	77	7.2							
28	13004	0	602*	1350	91	6.8							
30	15217	0	204	2012	111	6.8							

Soil 5190							Soil 5187					
Weeks	SO$_4$	CO$_3$	HCO$_3$	Ca	Mg	pH	Weeks	SO$_4$	CO$_3$	HCO$_3$	Ca	Mg	pH
0	3622	211	409	18	0	9.2	0	2535	144	256	8	0	8.2
2	4418	140	312	12	0	8.8	2	6518	0	306	1214	101	7.4
4	4612	124	210	22	0	8.8	4	14702	0	184	4421	178	7.0
6	4900	106	374	16	0	8.8				•			
8	5166	52	291	34	0	8.4							
10	5318	22	220	47	10	8.2							
12	5677	0	510	218	21	7 8							
14	6019	0	884	410	47	7.8							
16	6771	0	1214	656	52	7.6							
18	6944	0	1320	784	70	7.2							
20	7520	0	1200	1012	91	6.8							

* The extract gradually changed in color from a dark brown through a straw color to colorless in these samples.

The data show that as oxidation continued the content of sulfate increased while soluble carbonate decreased and the bicarbonate fluctuated considerably. This fluctuation in bicarbonate is quite striking in all the soils studied. In certain cases there was a distinct decrease in bicarbonate in the presence of normal carbonate. Since dilute acids convert carbonate into an equivalent amount of bicarbonate, the sum of these salts should be constant as long as the normal carbonate remains in solution. From the graph (fig. 1) plotted for one of these soils it is apparent that such a reciprocal relationship between carbonate and bicarbonate was not maintained

under these conditions. The lack of correlation between carbonate decomposition and bicarbonate formation occurred in varying degrees in all the soils studied.

Upon the neutralization of all of the soluble carbonate there was an immediate increase in the amount of soluble calcium, a result which is to be expected from the solubility of the calcium salts and is mentioned at this point because of the important relationship it bears to the use of sulfur, as brought out in later studies.
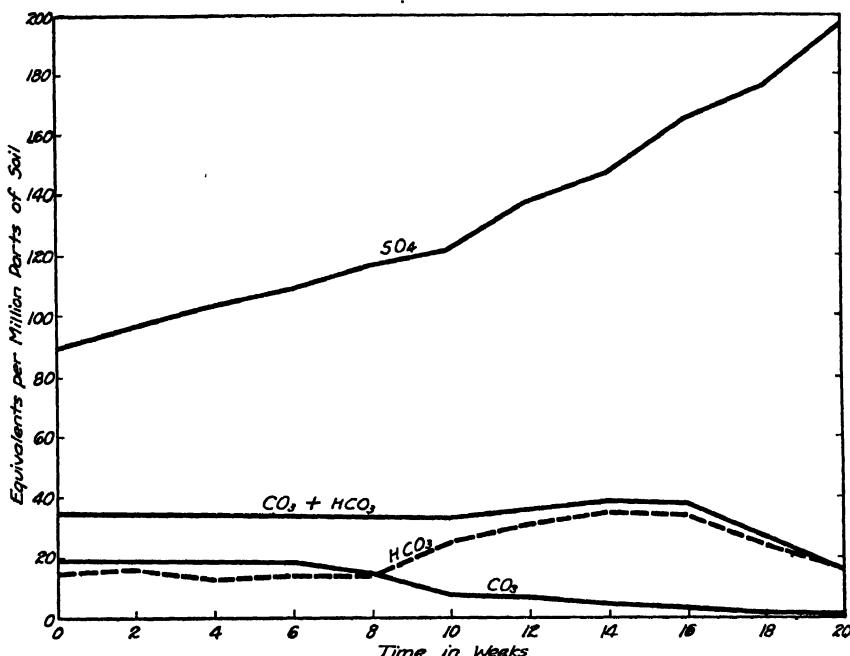
Fig. 1. The relation between the formation of sulfate and the decomposition of carbonate and bicarbonate in sulfofication experiments with soil 5189.

These experiments indicate clearly that there is no apparent relationship between the number of equivalents of sulfate formed and the number of equivalents of carbonate neutralized. The data from one series of analyses, shown graphically in figure 1, makes it evident that much more sulfate was formed than was necessary to account for the carbonate neutralized. In fact, an excess of sulfate was formed above the amount necessary to account for both the carbonate and the bicarbonate neutralized. This lack of close relationship between sulfate formation and the neutralization of carbonate and bicarbonate, as well as the simultaneous decomposition of both carbonates and bicarbonates, was clearly pointed out by Kelley and Thomas.[16]

These results and those of other workers, as well as the data obtained in field trials to be discussed later, indicate that sulfur will undergo relatively active oxidation in alkali soils. However, the anomalous decomposition of bicarbonate in the presence of normal carbonate and the inability to obtain even approximate correlations between sulfate formation and carbonate decomposition in the carefully controlled laboratory experiments seemed rather puzzling. Further experiments were accordingly made in an attempt to find a rational explanation of the chemical changes occurring within the soil. Since sulfur becomes oxidized to sulfuric acid and the chemical changes are due to the action of this acid, a study was made of the effects produced by the addition of solutions of sulfuric acid.

Alkaline Soils and Sulfuric Acid.—Dilute solutions of sulfuric acid were shaken with the soil in the ratio of 5 to 1. The shaking was continued for two hours, when the solutions were filtered through Pasteur-Chamberland filters. The first 250 cc. of filtrate was discarded and the remainder was analyzed by standard methods[14] for CO_3, HCO_3, Cl, SO_4, SiO_2, Na, K, Ca, and Mg. The accuracy of the bicarbonate titration is limited by the color change of methyl orange, which is not very distinct in the dark-colored soil extracts common to alkaline soils. The carbonate titration with phenolphthalein, however, is accurate despite the dark color, since the color change is pronounced. By calculating the difference between the total CO_2 and the CO_2-equivalent of the normal carbonate, an accurate bicarbonate determination was made possible. The total CO_2 was determined in 100 cc. of the extract by adding 50 cc. of 0.15N. HCl and absorbing the evolved CO_2 in 0.10 N KOH. The KOH was then titrated to the phenolphthalein end point and the CO_2 contained in the standard alkali subtracted from the total CO_2 figure thus obtained. Duplicate determinations checked within 0.1 cc. of 0.10 N KOH.

TABLE 3

COMPOSITION OF DILUTE H_2SO_4 EXTRACTS OF SOIL 5189

Parts per million of dry soil

Treatment	CO_3	HCO_3	CO_2	Cl	SO	SiO_2	Ca	Mg
H_2O	1105	1160	1635	8233	4257	76	42	20
0.0034 N H_2SO_4	650	1479	1625	8261	4989	74	74	20
0.0040 N H_2SO_4	480	1653	1645	8237	5200	57	82	22
0.0050 N H_2SO_4	288	2083	1630	8275	5351	49	102	18
0.0057 N H_2SO_4	267	2031	1620	8252	5445	64	142	21
0.0066 N H_2SO_4	120	2316	1625	8257	5687	75	151	20
0.0070 N H_2SO_4	Trace	2435	1640	8264	5831	64	192	21
0.0075 N H_2SO_4	0	2468	1645	8271	6052	68	254	28
0.0080 N H_2SO_4	0	2513	1630	8252	6152	67	258	44

TABLE 4

COMPOSITION OF DILUTE H_2SO_4 EXTRACTS OF SOIL 5189 AFTER LEACHING WITH WATER

Parts per million of dry soil

Treatment	CO_3	HCO_3	CO_2	Cl	SO_4	SiO_2	Ca	Mg	Na	K
H_2O	59	752	610	10	45	56	0	12	310	18
0.0005 N H_2SO_4	26	733	605	10	154	62	0	11	342	44
0.0010 N H_2SO_4	9	740	635	12	263	55	0	10	381	56
0.0015 N H_2SO_4	0	729	625	10	421	55	23	9	402	78
0.0020 N H_2SO_4	0	800	615	12	531	54	66	9	414	91
0.0025 N H_2SO_4	0	843	635	10	652	57	95	10	426	103

TABLE 5

COMPOSITION OF DILUTE SULFURIC ACID EXTRACTS OF SOIL 1869

Parts per million of dry soil

Treatment	CO_3	HCO_3	CO_2	Cl	SO_4	SiO_2	Ca	Mg	Na	K
H_2O	141	589	570	52	47	43	24	12	307	48
0.0005 N H_2SO_4	99	613	555	50	162	47	26	12	335	68
0.0010 N H_2SO_4	48	741	550	50	281	46	25	12	371	81
0.0015 N H_2SO_4	0	851	590	48	369	50	27	11	407	90
0.0020 N H_2SO_4	0	833	575	49	486	47	52	12	425	94
0.0025 N H_2SO_4	0	827	575	48	583	46	75	14	451	100

TABLE 6

COMPOSITION OF DILUTE SULFURIC ACID EXTRACTS OF SOIL 5696

Parts per million of dry soil

Treatment	CO_3	HCO_3	CO_2	Cl	SO_4	SiO_2	Ca	Mg	Na	K
H_2O	336	497	395	4827	1774	97	28	16	4097	20
0.0005 N H_2SO_4	312	479	385	4834	1915	88	28	13	4160	31
0.0010 N H_2SO_4	273	625	385	4823	2077	87	28	12	4195	49
0.0015 N H_2SO_4	258	653	390	4889	2217	74	30	12	4271	68
0.0020 N H_2SO_4	204	708	410	4856	2276	69	31	14	4337	77
0.0025 N H_2SO_4	180	805	440	4879	2330	69	35	13	4376	91
0.0030 N H_2SO_4	189	817	470	4828	2381	66	42	18	4307	86
0.0035 N H_2SO_4	144	1079	595	4831	2496	62	46	17	4399	100
0.0040 N H_2SO_4	135	1135	610	4885	2646	63	47	21	4425	121
0.0045 N H_2SO_4	123	1186	660	4920	2729	64	49	22	4583	138
0.0050 N H_2SO_4	123	1226	690	4855	2354	61	56	27	4646	144
0.0055 N H_2SO_4	102	1369	850	4831	2983	60	68	31	4700	169
0.0060 N H_2SO_4	Trace	1781	1020	4838	3726	90	132	37	5284	188
0.0150 N H_2SO_4	0	2925	1860	4811	5456	128	321	56	6187	234
0.0250 N H_2SO_4	0	3577	2300	4873	7116	157	779	68	6531	287

TABLE 7

COMPOSITION OF DILUTE SULFURIC ACID EXTRACTS OF SOIL 5190

Parts per million of dry soil

Treatment	CO₃	HCO₃	CO₂	Cl	SO₄	SiO₂	Ca	Mg	Na	K
H₂O	162	991	810	61	54	45	16	15	530	32
0.0005 N H₂SO₄	120	1095	805	63	167	64	19	13	560	44
0.0010 N H₂SO₄	120	1020	800	56	286	73	19	13	620	56
0.0015 N H₂SO₄	71	1275	825	55	407	51	24	16	660	61
0.0020 N H₂SO₄	58	1354	900	54	528	45	25	20	691	80
0.0025 N H₂SO₄	53	1394	925	57	657	43	30	23	744	87
0.0030 N H₂SO₄	44	1450	955	61	775	43	34	27	971	90
0.0035 N H₂SO₄	38	1566	1045	57	873	45	57	40	848	98
0.0040 N H₂SO₄	33	1670	1130	55	972	46	83	44	899	100
0.0045 N H₂SO₄	21	1724	1220	57	1036	51	89	57	931	101
0.0050 N H₂SO₄	10	1800	1295	60	1196	53	100	70	1013	99
0.0055 N H₂SO₄	Trace	1841	1360	70	1297	49	102	74	1057	103
0.0060 N H₂SO₄	Trace	1880	1460	68	1429	54	135	83	1110	106
0.0065 N H₂SO₄	Trace	1991	1540	64	1551	64	142	96	1176	104
0.0070 N H₂SO₄	Trace	2036	1605	66	1681	69	170	104	1238	100

The data in tables 3, 4, 5, 6, and 7 were obtained from soils 5189, 5190, 5696 (a Lahontan clay from Fallon, Nevada), and 1869 (a Fresno fine sandy loam from Kearney Ranch, Fresno, California). Soils 5189 and 5190 were leached to remove the excess of soluble sodium salts, which interfere with an accurate determination of sodium.

Under these conditions the effect of sulfuric acid was very different from that which takes place in a soil during sulfur oxidation. The addition of sulfuric acid always resulted in a decrease in normal carbonate and an approximately equivalent increase in bicarbonate. In every soil to which increasing amounts of sulfuric acid were added, the following regular changes were noted up to the point where soluble normal carbonate disappeared: (1) A decrease in normal carbonate and a reciprocal increase in bicarbonate; (2) an increase in sulfate equivalent to that added as H_2SO_4; (3) an increase in soluble sodium and potassium with every increase in the concentration of sulfuric acid; (4) not more than a slight increase in calcium and magnesium until the normal carbonate was greatly reduced, but with the neutralization of all of the normal carbonate there was an immediate increase in soluble calcium and magnesium; (5) only slight changes in soluble SiO_2.

In order to show the possible reactions produced by the acid, the data in the above tables have been recalculated on the basis of the following assumptions:

First, that the decrease in carbonate is accounted for by the equation:

$$2\,Na_2CO_3 + H_2SO_4 = Na_2SO_4 + 2\,NaHCO_3.$$

Second, that the increase in soluble sodium and potassium is accounted for by one or more of the following type equations:

(1) $\quad 2\,NaX + H_2SO_4 = 2\,HX + Na_2SO_4$

(2) $\quad 2\,CaCO_3 + H_2SO_4 = CaSO_4 + Ca(HCO_3)_2$

$$4\,NaX + CaSO_4 + Ca(HCO_3)_2 = 2\,CaX_2 + Na_2SO_4 + 2\,NaHCO_3.$$
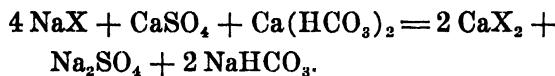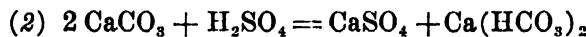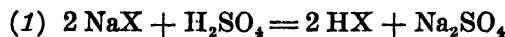
Third, that any increase in calcium and magnesium was due either to the solubility of $CaCO_3$, $MgCO_3$ or silicates in the saline solution, or to the following reaction:

$$CaX_2 + H_2SO_4 = 2\,HX + CaSO_4.$$

The foregoing data calculated on the basis of these assumptions are shown in tables 8, 9, 10, and 11.

TABLE 8

THE EFFECT OF SULFURIC ACID ON SOIL 5189, CALCULATED FROM THE DATA OF TABLE 4

Expressed as equivalents per million parts of soil

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Na_2CO_3 neutralized	Replaced		Total of (1), (2), and (3)	H_2SO_4 added	Difference between (4) and (5)	Calcium brought into solution
	Na	K				
0.55	1.40	0.65	2.60	2.50	+0.10	0.00
0.83	3.10	1.00	4.93	5.00	−0.07	0.00
1.00	4.00	1.50	7.65	7.50	+0.15	1.15
1.00	4.52	1.82	10.64	10.00	+0.64	3.30
1.00	5.00	2.17	12.92	12.50	+0.42	4.75

TABLE 9

THE EFFECT OF H_2SO_4 ON SOIL 1869, CALCULATED FROM THE DATA OF TABLE 5

Expressed as equivalents per million parts of soil

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Na_2CO_3 neutralized	Replaced		Total of (1), (2), and (3)	H_2SO_4 added	Difference between (4) and (5)	Calcium brought into solution
	Na	K				
0.70	1.22	0.50	2.42	2.50	−0.08	0.00
1.55	2.78	0.80	5.13	5.00	+0.13	0.00
2.35	4.35	1.05	7.75	7.50	+0.25	0.00
2.35	5.13	1.15	10.03	10.00	+0.03	1.40
2.35	6.26	1.30	12.46	12.50	−0.04	2.55

TABLE 10

THE EFFECT OF H_2SO_4 ON SOIL 5696, CALCULATED FROM THE DATA OF TABLE 6

Expressed as equivalents per million parts of soil

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Na_2CO_3 neutralized	Replaced		Total of (1), (2), and (3)	H_2SO_4 added	Difference between (4) and (5)	Amount of calcium brought into solution which in turn replaced Na
	Na	K				
0.40	2.74	0.27	3.41	2.50	+0.91	0.0
1.05	4.26	0.62	5.93	5.00	+0.93	0.0
1.30	7.58	1.20	10.08	7.50	+2.58	0.0
2.20	10.43	1.42	14.05	10.00	+4.05	0.0
2.51	12.10	1.78	16.39	12.50	+3.89	0.8
2.45	9.13	1.65	13.23	15.00	−1.77	1.3
3.27	13.13	· 2.00	18.40	17.50	+0.90	4.0
3.35	14.26	2.50	20.11	20.00	+0.11	4.1
3.55	21.10	2.95	27.60	22.50	+5.10	5.2
3.55	23.95	3.10	30.60	25.00	+5.60	5.5
3.90	26.20	3.72	33.82	27.50	+6.32	9.7
5.60	51.50	4.20	61.30	40.00	+21.30	10.7
5.60	90.90	5.10	101.60	75.00	+26.60	24.3
5.60	106.00	6.60	118.20	125.00	−6.80	22.4

TABLE 11

THE EFFECT OF H_2SO_4 ON SOIL 5190, CALCULATED FROM THE DATA OF TABLE 7

Expressed as equivalents per million parts of soil

(1)	(2)	(3)	(4)	(5)	(6)	(7)
Na_2CO_3 neutralized	Replaced		Total of (1), (2), and (3)	H_2SO_4 added	Difference between (4) and (5)	Amount of calcium brought into solution which in turn replaced Na
	Na	K				
0.70	1.30	0.30	2.30	2.50	−0.20	0.0
0.70	3.90	0.60	5.20	5.00	+0.20	0.0
1.50	5.60	0.70	7.80	7.50	+0.30	0.0
1.73	7.00	1.20	9.93	10.00	−0.07	1.7
1.81	9.30	1.40	12.51	12.50	+0.01	1.6
1.97	11.40	1.50	14.87	15.00	−0.03	2.3
2.07	13.90	1.70	17.67	17.50	+0.17	3.3
2.15	16.00	1.70	19.85	20.00	−0.15	4.5
2.35	18.40	1.80	22.55	22.50	+0.05	6.0
2.54	20.70	1.70	24.94	25.00	−0.06	6.6
2.70	22.90	1.80	27.40	27.50	−0.10	7.8
2.70	25.20	1.80	29.70	30.00	−0.30	9.0
2.70	28.10	1.80	32.60	32.50	+0.10	10.1
2.70	30.70	1.70	35.10	35.00	+0.10	10.4

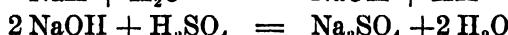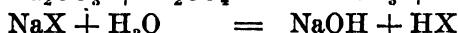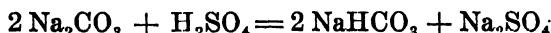By means of these assumptions, which are based upon our present theories concerning the origin and properties of alkaline soils, it is possible, with one exception, to account almost quantitatively for all of the sulfuric acid that was added to these soils. The recalculation of the data in this way makes possible a much clearer picture of the possible reactions between the acid and the alkaline materials of the

soil. Thus in accounting for the 2.50 equivalents of acid added to soil 5190 it is seen that 0.70 equivalents reacted with sodium carbonate, 1.30 equivalents was exchanged for sodium and 0.30 equivalents was exchanged for potassium.
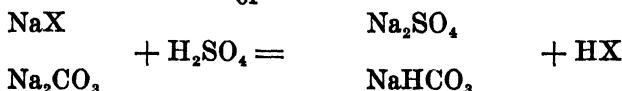
Upon approaching the complete neutralization of Na_2CO_3 in soil 5696, the increase in soluble sodium was greatly in excess of the theoretical amount. This soil differs from the other soils studied in that it contains a high concentration of soluble salts. It is also high in calcium carbonate. The salinity greatly complicates the analytical determination of sodium and potassium, a fact which may in part account for the results obtained. The extreme divergence upon the approach of complete neutralization of all of the normal carbonate is, however, greatly in excess of any possible analytical error.

It is known that neutral sodium salts increase the solubility of $CaCO_3$. The gradually increasing amounts of calcium found as the neutralization of carbonate proceeded was probably due in part at least to this type of solution. Moreover, if it is assumed that the sulfuric acid reacts with calcium carbonate as represented by the equation $2\,CaCO_3 + H_2SO_4 = Ca(HCO_3)_2 + CaSO_4$, it is apparent that one equivalent of acid may bring two equivalents of calcium into solution. This calcium may then be exchanged for sodium and thus result in the bringing of two equivalents of sodium into solution for one equivalent of acid. Accordingly any attempt to account quantitatively for the sulfuric acid added under these conditions is considerably complicated.

The Partition of the Added Acid.—The data show that the amount of acid necessary to effect the neutralization of an alkali soil may be much greater than the theoretical figure that is indicated by the amount of soluble carbonate present. Two changes were noted with the first increments of acid added. Each increment decreased the concentration of sodium carbonate and increased the concentration of soluble sodium. The reaction may be considered as an immediate reaction between sodium carbonate and sulfuric acid followed by a further hydrolysis, or it may be considered as a partitioning of the acid as illustrated by the following:

$$2\,Na_2CO_3 + H_2SO_4 = 2\,NaHCO_3 + Na_2SO_4$$
$$NaX + H_2O = NaOH + HX$$
$$2\,NaOH + H_2SO_4 = Na_2SO_4 + 2\,H_2O$$

<center>or</center>

$$\begin{matrix} NaX \\ Na_2CO_3 \end{matrix} + H_2SO_4 = \begin{matrix} Na_2SO_4 \\ NaHCO_3 \end{matrix} + HX$$

The equilibrium is the same in either case and it is of slight significance which reaction is favored. A partitioning of the acid, that is, an immediate neutralization of both the alkaline silicate complexes and the sodium carbonate more clearly emphasizes the degree of alkalinity of the two compounds. The greater portion of the sulfuric acid will react with the more alkaline compound, in this case the alkaline silicate complex. The active neutralization of the acid by the silicate complex is in a measure a verification of the potential presence of NaOH in the soil, as was experimentally shown by Cummins and Kelley.[4]

The following data show the amount of acid required to neutralize the soluble sodium carbonate in a 1–5 water extract of the soil, and the total amount of acid which it was necessary to add to the soil in order to neutralize both the alkaline silicates and the sodium carbonate.

TABLE 12

DATA SHOWING THE RELATION BETWEEN SODIUM CARBONATE AND TOTAL ALKALINITY OF SOILS

	Equivalents of acid required to neutralize	
	Na_2CO_3	Total alkalinity
Soil 5190 (leached)......	2.70	35.00
Soil 5696 (unleached)	5.60	40.00
Soil 5189 (leached).......	1.00	7.50
Soil 5189 (unleached)	18.50	35.00
Soil 1869 (leached)....	2.35	7.50

The data of table 12 show that the amount of sodium carbonate present is no necessary indication of the amount of acid required to neutralize all of the alkalinity of the soil. Although the reactions which take place in alkali soils as sulfur is oxidized differ somewhat from those produced by a solution of sulfuric acid, it seems certain that the amount of sulfur required in the practical treatment of an alkali soil may greatly exceed the sulfur equivalent of its soluble carbonate and bicarbonate content. In the case of soil 5189, it is also shown that the leaching with water greatly reduced both the soluble and the total alkalinity. The latter was reduced by leaching alone from 35 to 7.5 equivalents of acid. The advisability of such leaching in the reclamation af an alkaline soil is, however, very doubtful, since a large part of the organic matter and the colloidal material will be washed down along with the salts, and the remaining soil may then be mainly a sand unsuited to the growth of crops. There is also an unnecessarily large loss of plant nutrients, as emphasized by Hilgard and others.

Rôle of Hydrogen Ions in the Neutralization of Soil Alkalinity.—
In considering alkaline soils it has been found desirable to separate
them into two classes; namely those high in calcium carbonate and
those low in calcium carbonate. The exact amounts which determine
this classification have not been considered in the present paper. The
samples obtained from the Kearney Ranch, soils 1869 and 5189, are
low in calcium carbonate. The addition of acid to these soils up to
the point of converting all of the sodium carbonate into sodium
bicarbonate yielded no increase in total CO_2 in the extract. From this
result it is concluded that calcium carbonate was not involved in the
reaction. Furthermore, since no significant change was noted in the
concentration of the soluble parts other than Na and K, it must be
assumed that the only replacing agent was the H ion. Such a state-
ment does not imply, however, that the complete neutralization of an
alkaline soil would result in a soil saturated mainly with H ions.

It was also found that the increase in calcium brought into solution
in these two soils upon neutralization of all of the sodium carbonate
was not accompanied by an increase in CO_2. Therefore, the con-
clusion is drawn that the source of this dissolved calcium was mainly
silicates. This further emphasizes the importance of calcium car-
bonate in the application of sulfur or sulfuric acid to an alkaline soil,
for in the absence of $CaCO_3$, H ions not only replace sodium from
the silicate complex, but may also bring calcium into solution from
the limited supply of calcium silicate present. The sulfuric acid will
thus further impoverish the soil of its calcium. This conclusion is in
harmony with Gedroiz' findings upon the energy of absorption of
H ions as previously mentioned.

In the presence of calcium carbonate the equilibrium conditions
are very different. The first few additions of acid yielded results
similar to those found in the absence of calcium carbonate. With
further additions there was an increase in the total CO_2 extracted and
an increase in soluble calcium. The increase in soluble calcium took
place in the presence of soluble carbonate and is due to the greater
solubility of $CaCO_3$ in the increasingly saline solution. However, the
increase in CO_2 was much greater than the increase in soluble calcium.
The difference between the calcium carbonate dissolved and the CO_2
found is assumed to represent the amount of calcium which was first
brought into solution from calcium carbonate but which then served
to replace sodium* from the silicate complex and thus passed out of

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* Throughout this discussion the terms ''sodium and ''calcium'' are used
without mention of potassium and magnesium, which are similarly involved in
all of these changes to a lesser extent. It is not intended to imply their absence
in the reactions, but these words are simply so used to avoid the mention of
both monovalent or divalent salts each time they are considered.

solution. The data showing the approximate extent of this replacement are given in tables 10 and 11. The accuracy of these calculations is limited by the uncertainty as to what extent magnesium acted as a replacing agent. However, the error is of no significance in the present case, since the intent is simply to indicate the relative extent to which hydrogen ions and calcium acted as replacing agents in these soils. If the results are subtracted from the total calcium and magnesium as shown in tables 6 and 7, it is possible to gain an approximate idea of the extent to which hydrogen and calcium have displaced sodium.

These results indicate that even in the presence of large amounts of calcium carbonate the replacement of Na was effected mainly by H ions. Upon the complete neutralization of the sodium carbonate the proportion of hydrogen ions which served to replace sodium decreased, and the amount of calcium which acted as a replacing agent for sodium increased. The exact amounts of replacement brought about by calcium and hydrogen ions after all of the sodium carbonate was neutralized cannot be determined with accuracy, since a small loss of carbon dioxide took place. Since the calcium in solution rapidly increased, it seems probable that it would ultimately predominate as a replacing agent.

No extended study was made on the physical state of the soils as affected by these treatments. Several series of cylinder experiments on the rate of settling of the sulfuric-acid-treated soils indicate that upon the replacement of sodium there was a marked flocculation of the soil particles. The rate of settling of the soils before filtration was always more rapid and more complete where the larger amounts of acid were used. Rudolfs[22] reported similar results in soils in which sulfur oxidation had taken place. Whether the flocculating action was due to H ions cannot be stated, since with sufficient replacement of Na by H ions to observe this change, there was always an increase in calcium in the solution. The flocculating action of Ca, as already stated, is very great.

Alkaline Soils and Calcium Sulfate.—It has already been pointed out that upon the addition of sulfuric acid to alkaline soils the equilibrium is not the same as that resulting from sulfur oxidation. The effect of calcium sulfate seems to offer, in part, an explanation for this difference.

The same procedure was followed in these studies as with the sulfuric acid. A saturated solution of calcium sulfate was prepared and diluted to known concentrations and added to the soil. The results are reported in tables 13 and 14.

TABLE 13

DATA SHOWING THE DIFFERENCE PRODUCED IN SOIL 5190 BY ADDING EQUIVALENT
AMOUNTS OF CaSO₄ AND H₂SO₄

Parts per million

Treatment	CO₃	HCO₃	CO₂	Cl	SO₄	SiO₂	Ca	Mg	Na	K
H₂O	162	991	810	61	54	45	16	15	530	32
0.0025 N H₂SO₄	53	1344	900	57	657	43	30	23	731	87
0.0025 N CaSO₄	150	682	600	58	621	47	27	41	552	42
0.0050 N H₂SO₄	10	1614	1295	60	1196	53	120	80	893	99
0.0050 N CaSO₄	132	510	505	55	1141	61	66	68	634	51
0.0070 N H₂SO₄	Trace	1978	1605	66	1681	69	270	144	968	100
0.0070 N CaSO₄	80	344	450	61	1659	54	136	86	708	42

TABLE 14

DATA SHOWING THE DIFFERENCE PRODUCED IN SOIL 5696 BY ADDING EQUIVALENT
AMOUNTS OF CaSO₄ AND H₂SO₄

Parts per million

Treatment	CO₃	HCO₃	CO₂	Cl	SO₄	SiO₂	Ca	Mg	Na	K
H₂O	336	497	395	4827	1724	97	28	16	4097	20
0.0025 N H₂SO₄	180	805	440	4879	2330	69	35	13	4376	91
0.0025 N CaSO₄	356	189	180	4832	2178	80	46	30	4104	84
0.0050 N H₂SO₄	123	1226	690	4855	2854	61	56	27	4646	144
0.0050 N CaSO₄	326	207	145	4888	2749	62	72	35	4347	121
0.0150 N H₂SO₄	0	2925	1860	4811	5456	128	321	56	6187	224
0.0150 N CaSO₄	310	85	100	4907	5291	61	160	39	5382	194

A large part of the added $CaSO_4$ was precipitated as $CaCO_3$. The remainder replaced sodium from the silicate complex. The precipitation of $CaCO_3$ was evident from the marked decrease in the concentration of CO_2 in the extract. Upon precipitation of calcium carbonate the corbonate-bicarbonate equilibrium of the soil was disturbed, and in the presence of alkaline silicate complexes and the products of their hydrolysis a portion of the bicarbonate was converted into carbonate. Thus the precipitation of normal carbonate reduced the concentration of bicarbonates, owing to the alkalinity of the silicate complexes. That these silicate complexes are a primary source of alkalinity is evident, since the concentration of sodium carbonate was only slightly decreased by large additions of calcium sulfate.

The data show that calcium sulfate is not nearly as effective as sulfuric acid in the replacement of sodium from the silicate complex. This is due to differences in the type of reaction. Calcium sulfate

functions, in considerable part, to precipitate the carbonate as calcium carbonate with the simultaneous formation of sodium sulfate. Sulfuric acid, on the other hand, only partially neutralizes the sodium carbonate by forming the bicarbonate. Consequently a given amount of sulfuric acid removed a greater amount of the normal carbonate than did an equivalent amount of calcium sulfate.

Absorption of Carbon Dioxide by Soil Organisms.—If it is assumed that the process of sulfur oxidation in alkaline soils is in part a localized reaction around particles of calcium carbonate, it is possible to explain the apparently anomalous carbonate-bicarbonate results of the sulfur-oxidation experiments. The organisms are autotrophic and by means of a localized activity around $CaCO_3$ particles they may obtain the carbon necessary for their growth, and in the presence of calcium carbonate the end product of the reaction, sulfuric acid, is neutralized. The calcium sulfate formed during this reaction, upon diffusion from these localized zones, would in turn be again precipitated as calcium carbonate. This reaction would affect the carbonate-bicarbonate equilibrium to the extent to which CO_2 was lost from the system, somewhat as was the case when $CaSO_4$ was added.

Under the conditions that exist in the soil during sulfur oxidation, a decrease in bicarbonate involves either the changes hypothecated or a loss of carbon dioxide to the air. Since the results presented were obtained in soils containing sodium carbonate, the loss of carbon dioxide to the air should have been very small. Whether calcium carbonate plays such a rôle in sulfur oxidation or not, its presence in large amounts in an alkaline soil may be regarded as potentially valuable in their reclamation by means of sulfur, since it affords a source of calcium which the products of sulfur oxidation may bring into solution and thus make available as a plant nutrient, besides effecting the substitution of sodium in the exchange complex.

Tank Experiments.—Galvanized iron containers 19 inches in diameter and 24 inches in height were used in these experiments. Two hundred and fifty pounds of soil was added to each container, and placed upon a layer of broken granite to permit drainage through an opening in the bottom. Soils 5189 and 5190 were used. Six cans of each soil were set up, and each of the following treatments was applied to one can of each set: 100 grams sulfur after the soil was thoroughly leached; 100 grams sulfur without previous leaching; 100 grams sulfur and 85 grams calcium carbonate; 100 grams sulfur and 116 grams calcium sulfate; sulfuric acid equivalent to 100 grams sulfur applied as 0.05 N solution. The amounts of the several

treatments correspond to those used in the field experiments referred to later. The materials were thoroughly mixed with the surface 6 inches of soil on March 15, 1924, and allowed to stand with occasional irrigations and cultivations. Samples were drawn from the first foot and the second foot on May 10, 1924, and October 10, 1924. The soils were leached with water on November 26, 1924, and again sampled on January 2, 1925. Barley was planted on January 2, 1925, and the crop harvested May 6, 1925. The final sampling was made on June 12, 1925, after a very slight leaching made for the purpose of washing the products of sulfur oxidation down into the lower layers of the soil.

Practically all of the sodium carbonate was neutralized within fifteen months' time in the soils of every treated tank. With the rather large accumulations of soluble calcium in the second foot of the soil, it seems apparent that the neutralization must have taken place at the expense of a portion of the surface calcium, which was washed down in the leaching process. This loss of calcium was the more apparent in soil 5190, which is high in calcium carbonate. The same fluctuation in the bicarbonate and its decomposition took place in these tank experiments as in the sulfur-oxidation experiments.

Barley was planted in each of the soils on January 2, 1925. The seeds germinated in each of the twelve tanks, but at the end of two weeks the plants in the check tanks began drying up at the tips of the leaves and soon died. In the tanks receiving sulfur alone before and after leaching, the plants attained a height of about 6 inches and then died back from the tips of the leaves as did the check plants. In none of the tanks was a satisfactory growth obtained.

The best growth of the barley as judged by its appearance and final yield was on the Utah soil to which were added sulfur and calcium carbonate, and sulfur and calcium sulfate. This seemed rather surprising in view of the large amounts of calcium carbonate initially present in this soil. The best growth on the Fresno soil was where sulfur alone was applied and this was only slightly better than that produced by sulfur and calcium carbonate. Where sulfuric acid was added the growth of the barley was stunted, but the crop headed out and yielded an amount of grain slightly less than in those tanks receiving sulfur and calcium carbonate.

Field Experiments.—Five plots (21 to 25), each 40 by 135 feet, were staked out on one of the worst portions of the Kearney Vineyard Experimental Tract near Fresno, California, on May 10, 1923. The plots were given the following treatments: plot 21, 3000 pounds per

acre of sulfur and 4080 pounds of gypsum; plot 22, 3000 pounds per acre of sulfur and 3000 pounds of $CaCO_3$; plot 23, untreated; plots 24 and 25, 3000 pounds per acre of sulfur. Plot 25 was thoroughly leached with water by heavy flooding before the sulfur was applied, while the other plots were not flooded until several months after the treatments were applied. The materials were applied and plowed under on June 2, 1923. Before the experiments were begun thirteen samples were drawn from each plot to the following depths: 0–6 inches, 6–12 inches, 12–24 inches, 24–36 inches, and 36–48 inches. The samples were taken at 10-foot intervals along the center line of the plots, and later samples were drawn at places 6 inches distant from those of the original samples. A second set of samples was drawn on December 15, 1924, and a third on March 24, 1925, just after the plots had been thoroughly leached. Analyses were made on 1–5 water extracts of these samples.

The extreme variability of alkali soils greatly complicates the determination of the chemical changes occurring in a field experiment under any treatment. This has proved to be the case in these experiments. The brief period employed in these experiments is also a factor affecting the conclusions. The data obtained for the soluble carbonate and sulfate in the first foot, presented in tables 15, 16, 17, 18, and 19, indicate that a considerable amount of the alkalinity had been neutralized by each of the treatments.

TABLE 15

COMPOSITION OF PLOT 21, TREATED WITH SULFUR AND GYPSUM MAY 15, 1923

Parts per million

Sample	May 1923		Dec. 1924		Mar. 1925	
	CO₃	SO₄	CO₃	SO₄	CO₃	SO₄
1	0	305	0	126	0	68
2	180	528	69	468	0	110
3	270	530	135	62	180	21
4	285	573	0	1370	162	51
5	186	788	90	624	30	98
6	210	500	0	3480	87	200
7	186	466	0	600	0	336
8	342	492	180	378	72	80
9	444	760	220	758	171	114
10	609	713	0	518	150	47
11	540	620	27	414	156	132
12	429	524	120	330	199	90
13	558	791	39	346	0	100

TABLE 16

COMPOSITION OF PLOT 22, TREATED WITH SULFUR AND $CaCO_3$ MAY 15, 1923

Parts per million

Sample	May 1923		Dec. 1924		Mar. 1925	
	CO_2	SO_4	CO_2	SO_4	CO_2	SO_4
1	156	41	109	318	78	69
2	204	151	259	236	171	67
3	138	202	222	580	144	48
4	210	690	210	192	195	74
5	180	654	168	546	141	96
6	189	460	0	418	0	156
7	174	189	195	190	153	106
8	231	781	150	394	150	71
9	390	534	135	748	219	156
10	489	736	231	222	114	150
11	402	883	180	332	174	466
12	438	885	231	496	264	586
13	531	848	135	524	0	532

TABLE 17

COMPOSITION OF UNTREATED PLOT 23

Parts per million

Sample	May 1923		Dec. 1924		Mar. 1925	
	CO_2	SO_4	CO_2	SO_4	CO_2	SO_4
1	210	171	150	176	24	30
2	180	716	330	392	249	16
3	120	431	240	534	354	49
4	300	603	330	380	300	30
5	180	653	270	358	330	20
6	130	655	90	624	171	59
7	141	86	150	456	216	44
8	150	1317	135	654	306	122
9	405	655	135	1014	390	292
10	360	602	240	540	366	41
11	270	696	345	384	429	116
12	420	790	375	734	330	38
13	390	524	285	114	240	33

TABLE 18

COMPOSITION OF PLOT 24, TREATED WITH SULFUR MAY 15, 1923

Parts per million

Sample	May 1923		Dec. 1924		Mar. 1925	
	CO_2	SO_4	CO_2	SO_4	CO_2	SO_4
1	330	328	24	578	30	136
2	200	347	0	834	138	126
3	390	461	156	392	216	130
4	261	379	30	660	288	276
5	210	108	0	592	0	246
6	240	107	63	1044	0	126
7	290	330	150	138	126	92
8	291	264	72	1040	156	79
9	510	2173	240	1134	240	302
10	354	294	210	2243	174	412
11	216	716	54	1354	0	272
12	150	633	165	342	120	130
13	171	464	74	624	24	130

TABLE 19

COMPOSITION OF PLOT 25, TREATED WITH SULFUR MAY 15, 1923, AFTER FIRST
LEACHING THE SOIL

Parts per million

Sample	May 1923		Dec. 1924		Mar. 1925	
	CO_2	SO_4	CO_2	SO_4	CO_2	SO_4
1	330	313	0	1228	30	108
2	150	72	165	268	168	78
3	300	255	300	262	264	184
4	270	170	180	618	285	286
5	230	174	225	1332	270	116
6	180	95	195	300	465	523
7	300	577	21	646	126	64
8	201	106	30	340	159	42
9	702	1014	120	1022	54	67
10	465	306	75	840	234	56
11	360	514	114	482	132	42
12	270	994	24	704	180	61
13	189	309	66	244	30	86

The first indication of effect from the treatments was shown by the rate at which the soil absorbed water upon flooding. All of the treated plots had become reasonably permeable to water when leached. The dikes one foot high around each plot were twice filled with water, all of which was absorbed within 48 hours in the case of the treated plots. The check plot, No. 23, on the other hand, remained quite impervious; much of the water stood on the surface at the end of one week and it was finally necessary to tap off the pools of water by surface drains.

Hubam clover was planted on all the plots in April, 1925. The crop response was just as striking as was the physical change in the soil, and the growth obtained shows that the treatments have more greatly affected the soil than is indicated by the analysis. By October, 1925, the Hubam clover had produced an excellent growth on all of the treated plots, whereas it was an entire failure on the check plot.

The difficulty of interpreting the chemical analyses of these samples accords well with the ideas presented earlier in this paper. It is evident from the equilibrium studies presented above that the neutralization of a large part of the alkaline compounds of a soil may be effected without this result being shown by a determination of soluble carbonate. The determination of the extent of sulfur oxidation is also complicated by the large amounts of sulfates already present in the soil, which tend to move by capillarity as a result of seasonal climatic changes.

These difficulties may be overcome, however, after the lapse of time, as is shown by the results obtained from another experiment in the same field where sulfur was applied at the rate of 3500 pounds per acre on May 24, 1921. This experiment is being conducted by the Chemistry Department of the Citrus Experiment Station, and the results have been made available to the writer. The method of sampling the soil was similar to that previously mentioned. The analyses of the original samples were made by Mr. S. M. Brown and are presented for the first and fourth-foot depths only (table 20).

TABLE 20

EFFECT OF SULFUR ON FRESNO ALKALI SOIL

	First foot								Fourth foot							
	Before treatment				2 years after treatment				Before treatment				2 years after treatment			
Sample	CO_3	SO_4	Ca	pH	CO_3	SO_4	Ca	pH	CO_3	SO_4	Ca	pH	CO_3	SO_4	Ca	pH
1...........	195	509	0	10	0	279	77	6.8	45	160	0	9.1	70	430	18	8.3
3...........	253	23	0	10	0	1955	546	6.9	45	44	0	9.2	108	554	0	8.4
5	375	216	0	10	0	469	133	7.0	150	54	0	9.6	168	649	0	8.4
7...........	200	45	0	10	0	215	78	6.9	150	44	0	9.6	0	209	60	8.2
9...........	285	15	0	10	0	514	175	6.9	270	95	0	9.6	18	232	0	8.2
11...........	345	370	0	10	30	669	79	8.4	105	46	0	9.6	75	385	25	8.5
13...........	300	417	0	10	0	2024	311	7.2	30	40	0	9.0	78	584	80	8.4
15...........	345	169	0	10	21	1477	193	8.4	30	44	0	9.1	0	198	45	8.2
17...........	255	242	0	10	30	1676	210	8.4	105	39	0	9.6	150	171	21	8.4
19...........	375	618	0	10	40	421	62	8.3	210	73	0	9.6	86	406	0	8.4
21...........	435	381	0	10	10	904	117	8.2	30	62	0	9.0	0	266	32	8.2
23...........	540	884	0	10	0	592	133	7.0	465	72	0	9.6	330	192	9	8.5
25...........	510	610	0	10	0	1978	348	7.1	90	56	0	9.0	174	593	7	8.6

It is evident that the oxidation of sulfur has affected the soil of this plot to a marked extent, especially in the first foot. The effect has also extended into the fourth foot to some extent, showing that it is possible to affect this soil to a considerable depth by the application of sulfur.

The crop records obtained from this plot show that after the lapse of four years' time the effect from the application of sulfur was very striking. Although the effect was slow in manifesting itself, owing no doubt to the fact that the oxidation of sulfur is a biological process in which the time element is of considerable importance, the growth of alfalfa, four years after the sulfur was applied, was excellent.

SUMMARY

A brief review of the investigations on the replacement of bases in soils, the relation of base exchange to alkaline soils and the oxidation of sulfur, is presented in this paper.

Sulfur oxidation took place very readily in alkaline soils under laboratory, greenhouse, and field conditions, and was most rapid in sandy soils and in the presence of sodium carbonate. Carbonates and bicarbonates were decomposed and bicarbonates and sulfates formed during sulfur oxidation, but no stoichiometric relationship was found between these processes.

The fact that the oxidation of sulfur brings about a simultaneous decomposition of carbonate and bicarbonate was investigated by studying the equilibrium between dilute sulfuric acid and calcium sulfate, and alkaline soils. The results showed that upon adding dilute sulfuric acid the amount of soluble sodium and potassium increased and sodium carbonate decreased, with only slight changes in the concentration of silica. With the approach of complete neutralization of the sodium carbonate, the concentration of calcium and magnesium increased. By assuming an exchange of hydrogen ions, stoichiometric with the increase in soluble sodium, potassium, calcium, and magnesium, it was possible to quantitatively account for approximately all of the acid added. Hydrogen ions were found to function as a replacing agent to a greater extent than calcium, even though considerable amounts of calcium were made soluble.

The addition of dilute sulfuric acid effects a partial neutralization of carbonate to the bicarbonate stage, while calcium sulfate brings about a precipitation of carbonate in the form of calcium carbonate. Hence, equivalent for equivalent, sulfuric acid is a much more efficient neutralizing material than calcium sulfate. In the light of the data

obtained from sulfofication studies, on the one hand, and equilibrium studies, on the other, it is hypothecated that sulfur undergoes oxidation around the particles of calcium carbonate, forming calcium sulfate, and that the organisms concerned utilize more or less of the carbon dioxide thus formed as a source of carbon.

Results are presented of tank and field trials in which the greater part of the alkalinity of the soil has been neutralized by the addition of sulfur, and in the field trials very striking improvements have resulted in the crop yields. The presence of calcium carbonate is very desirable in the reclamation of an alkaline soil by means of sulfur.

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THE ENZYMES OF PYTHIACYSTIS CITROPHTHORA Sm. AND Sm.[1-2]

L. JOSEPH KLOTZ[3]

I. INTRODUCTION

This paper reports a study of the enzymes produced by the fungus, *Pythiacystis citrophthora*, when grown in pure culture. For references and short general reviews of the several works on the enzymes of the various groups of fungi, see Waksman,[25] Waksman and Davison,[26] Dox,[6] Zeller[27] and Cooley.[5] Considering forms morphologically rather near to Pythiacystis, Emoto[7] found in species of Saprolegnia that amylase, inulase, raffinase, invertase, lactase, maltase, emulsin, salicase, glycolase, proteolytic enzymes (acid, neutral, alkaline), peroxidase and catalase were present as intra-cellular enzymes, and pectinase, cellulase, lipase, urease, tyrosinase and oxidase were absent. In the Achlya, amylase, inulase, cellulase, invertase, lactase, maltase, emulsin, salicase, proteolytic enzymes (acid, neutral, alkaline) peroxidase, and catalase were present and pectinase, glycolase, lipase, urease, tyrosinase and oxidase absent.

Among the enzymes reported by various workers as being produced by representatives of the Mucorales are cytase, cellobiase, pectinase, inulase, diastase, maltase, invertase, lactase, zymase, rennet and lipase. This briefly sums up the known work on the enzymes produced by the phycomycetous fungi.

II. MATERIAL AND METHODS

The organism used in the following work was a virulent strain of *Pythiacystis citrophthora* isolated by H. S. Fawcett of the Citrus Experiment Station and by him given the number 1309a. The pathogenicity of the fungus has been studied by Smith and Smith[21] in connection with the fruit rot of citrus and by Fawcett[8, 9] in connection with the gummosis disease. The virulence of this particular isolation was tested by hundreds of inoculations into the fruit and bark of various citrus species. It was grown for 10 days at room temperature (18–25° C) on glucose-potato-peptone broth made of the following materials:

1. Watery extract from 250 gm. of peeled, sliced and boiled potatoes
2. Dextrose, 20 gm.
3. Bacto-Peptone, 10 gm.
4. Water to make 1000 ml.

The mats were filtered on a Buchner funnel, washed with distilled water, and divided into two equal lots. One lot was desiccated and made permeable by treatment with acetone and ethyl ether according to the acetondauerhefe method of Albert, Buchner and Rapp.[1] See also Kohnstamm,[14] Onslow[16] (p. 23) and Dox[6] (p. 38). The coarse powder resulting was ground to a fine flour and stored in brown glass bottles. The other lot was not treated with acetone and ether but was dried in an electric oven at 45° C and then ground to a fine flour and added to the acetondauerhefe preparation, assuring the presence of enzymes that may have been destroyed by the first method. This material was the source of the enzymes throughout the work.

In the first experiment an extract was made of the fungus powder by grinding it with glass in twenty times its weight of distilled water. The suspension was allowed to extract for an hour, then filtered through filter paper, and finally through the finest Mandler filter candle. The last operation was done under aseptic conditions so that a sterile enzyme extract was obtained. This was added aseptically in 5-ml. quantities to 20 ml. of 1 per cent solutions of the various substrata, making use of a modification of the apparatus described by Smith.[21] In this series, therefore, it was unnecessary to add toluene, chloroform or other preservative. The enzyme "cultures" were incubated in the dark at 25° C for 8 days. The extract thus prepared showed a slight urease and maltase reaction, but no protease, no deaminase or deamidase with asparagin, and no invertase. Evidently only a small quantity of the enzymic materials passed the filter candle.

In all the subsequent work, unless otherwise specified, 20 cc. of 1 per cent solutions or suspensions of the various test materials were added directly to 250 mgm. of the enzyme powder in 200 ml. Erlenmeyer flasks, the system preserved with 1 ml. of C. P. toluene, and the flasks tightly stoppered. The "cultures" were incubated in the dark at temperatures ranging from 29° C to 39° C. For every culture prepared in this way a parallel check was run using enzyme powder that had been autoclaved at 16 pounds pressure for 15 minutes.

As further precautions blank determinations were made on extracts of the active and autoclaved powders as follows: (a) reducing power in Fehling's solution, (b) amino nitrogen (c) ammonia nitrogen, (d) total acidity and (e) active acidity. The various works on fungus enzymes have in general ignored the probability of an important difference between the powder before and after autoclaving. To determine the reducing power the iodometric method of Shaffer and Hartmann[20] was employed. Amino nitrogen was estimated by the van Slyke[24] micro method, ammonia nitrogen by Folin's aeration method [see Shaffer ('03)], and active acidity by the colorimetric method, using the indicators and buffers recommended by Clark.[4] Total acidity was determined by direct titration against standard alkali, phenolphthalein being used as an indicator. The reducing power is shown by the following determinations:

TABLE 1

REDUCTION OF FEHLING'S SOLUTION BY FUNGUS POWDER

Material	Incubation period (days)	Temperature (°C.)	Reducing power (mgm. Cu liberated by 1 ml. of extract)	
			Active	Autoclaved
.25 gm. enzyme powder+20 cc. H₂O +1 cc. toluol.	3	37	4.95	.6192
	4	39	3.16	.894
	10	38	6.88	.743
	21	38	4.599	.8256
	1½	40	2.614	.8944
	1½	40	2.924	.9976
	1½	40	3.37	.86 .

The inconsistencies in the above determinations are accounted for by the small reducing power of the extracts, which is below the minimum of 10 mgm. per ml. (or 1 mgm. per ml. when diluted to 50 ml.) recommended for accuracy by the authors. Small as these values are they are important because they negate what otherwise

would seem to be evidence for the presence of some specific carbo-hydrases in the enzyme powder.

Moreover, it is seen that autoclaving brings about a marked loss in the ability of the powder to reduce Fehling's solution. This is probably due in part to the destruction of the aldehyde groups of the dextrose absorbed by the mycelium; and is effected by the union of the aldehyde groups with the indole groups of the fungus proteins resulting in the formation of the dark-colored humin nitrogen. [See Gortner and Holm.[10]] The fungus powder was changed from a light gray to a dark brown by the autoclaving which may indicate that such a transformation had taken place.

TABLE 2

TOTAL AND ACTIVE ACIDITY, AND AMINO AND AMMONIA NITROGEN OF THE
ENZYME EXTRACTS

Incubation period	Temp.	Total acidity (ml. .098 N NaOH to neutralize 10 ml. of extract to phenolphthalein)		Active acidity (Pн)		Amino N (N₂ from 2 ml. ext.) ml.		Ammonia N (ml. .0952 N H₂SO₄ to neut. 5 cc. ext. to phenolphthalein)	
(*days*)	(°*C.*)	Active	Autoclaved	Active	Autoclaved	Active	Autoclaved	Active	Autoclaved
10	38	.45	.45	5.8	4.8	.44	.278	.35	.35

Taking these considerations into account it is evident that the auto-claved powder does not serve as a true check. Accordingly in the calculations, the reducing power of the active powder alone plus the reducing power of the substrate to which the autoclaved, inactivated powder had been added is substracted from the reducing power of the system with the active enzyme powder, and to this result is added the reducing power of the autoclaved enzyme. This reasoning is necessarily based upon the assumption that the catalytic effect of the fungus powder, other than its enzymic action, is similar in both the active and autoclaved powder. An example will illustrate the procedure.

Suppose

Mgm. Cu.

(1) Starch solution plus active enzyme gave a reduction 25

and (2) Active enzyme alone 5

and (3) Starch solution plus autoclaved enzyme 2

and (4) Autoclaved enzyme alone 1

The reduction due to the hydrolyzed starch is evidently not 25 minus 2 equal 23, but 25 minus 5 minus (2 minus 1) equals *19* if we make the assumption stated above. That assumption was made in the calculations of this work.

ESTERASES OR LIPASES

As test substrata for the determination of esterases, olive-oil emulsion, lemon oil emulsion, cream, methyl acetate, and ethyl acetate were used. The degree of change in active and total acidity was taken as the criterion for the presence or absence of these enzymes. A slight modification of the method of Rice and Markley[19] was used to test for lipase where cream was the substrate. The cream was heated to destroy any lipase present and then saturated with cane sugar as a preservative. The enzyme powder was added and the cultures incubated. The

TABLE 3

Action of Esterases of *Pythiacystis Citrophthora* on Various Substrates

Substrate	Incubation period (days)	Temp. (°C.)	Total acidity (Vol. .098N NaOH to neut. 10 ml. substrate)		Active acidity (Pн)	
			Active	Autoclaved	Active	Autoclaved
Olive oil	4	38	.30	.30	5.8	5.4
Lemon oil	4	38	1.00	1.05		
Cream	4	38	2.35	.85		
Methyl acetate	4	38	1.15	0.40	4.8	4.9
Ethyl acetate	4	38	1.20	0.45	4.5	4.9

acidity was determined at the beginning and at the end of incubation by diluting an aliquot with ten times its volume of water, and titrating to a phenolphthalein endpoint. The advantages of the method are pointed out by the authors. Cream is a well emulsified, natural fat, and the sugar preservative increases the viscosity and keeps the fat from separating for a long time. A disadvantage is an indistinct endpoint in the titration, but that is less troublesome if the aliquot is diluted. The principle of Bloor's[3] method, as described by Zeller[27] was used in preparing the olive oil emulsion. In this method 1 ml. of olive oil in 10 ml. of hot absolute alcohol was drawn into 100 ml. of cold distilled water by means of a suction flask fitted with a funnel having a capillary delivery end. The alcohol was then boiled off and the enzyme powder added. With the lemon oil no alcohol was used, the oil being sucked directly into the watery suspension of the enzyme powder. As usual toluol was used as a preservative.

The results show that the enzyme powder is capable of increasing the acidity of some of the ester substrates. This must be interpreted as being due to the production of fatty acids by hydrolysis. It may be

said here that none of these esters and oils used in 1 per cent strength in synthetic media having no other carbon source supported growth of the fungus. Cream was not tried in that way. Autoclaved cream alone did support growth. Lemon oil added to autoclaved rind and albedo was distinctly inhibitive to the growth of the organism.

CARBOHYDRASES

Table 4 shows the test materials used to demonstrate the presence or absence of the respective carbohydrases. The data for reducing power represent the values after the checks have been substracted according to the manner already described.

TABLE 4

Action of Fungus Powder on Various Carbohydrates

Substrate	Incubation period (*days*)	Temp. (°C.)	Reducing power (mgm. Cu liberated from Fehling's sol. by 1 ml. of substrate)
1. Lintner's soluble starch suspended in cold H₂O	4	39	2.335
2. Lintner's soluble starch suspension autoclaved	3	37	17.060
3. Lintner's soluble starch, autoclaved	1½	40	13.8976
4. Potato starch, cold H₂O	4	39	2.200
5. Potato starch suspension, autoclaved	4	39	16.579
6. Potato starch suspension, autoclaved	2	29	12.454
7. Inulin, cold H₂O	4	37½	.0402
8. Inulin, cold H₂O autoclaved	3	37	.9612
9. Inulin, cold H₂O	22	38	2.2684
10. Hemicellulose	12	25	2.578
11. Hemicellulose	23	25	3.9196
12. Hemicellulose	22	38	0.136
13. Cellulose	4	37½	0.00
14. Cellulose	16	38	0.00
15. Cellulose	22	38	0.00
16. Raffinose	3	37	4.4014
17. Lactose	2	29	2.6812
18. Lactose	3½	39	2.062
19. Maltose	2	29	8.667
20. Sucrose	2	29	40.2148
21. Sucrose	4	37	21.46*
22. Sucrose	1½	40	15.7208*
23. Sucrose	1½	40	4.9332†

* Different lot of enzyme powder used.

† Different lot of enzyme powder and a 10 per cent sucrose solution used.

Of the polysaccharides used (starch, inulin, hemicellulose, and cellulose) the first was the only one strongly hydrolyzed by the fungus powder, and this only after the starch had been gelatinized by heating in water. The Lintner's soluble starch and the potato starch behaved similarly. The presence of diastase is established. Inulin showed a slight reduction only after a long incubation with the powder. This may be partly explained by the low incubation temperature and low hydrogen ion concentration used. Pringsheim and Kohn[17] found the optimum temperature for the action of this enzym to be 55° C, and the optimum hydrion concentration to be P_H 3.8. No attempt was made to induce inulase activity in the organism by growing it in the presence of the carbohydrate. This phase was not considered with any of the test materials employed in this work. However, it is observed that peptone and starch were present in the nutrient in which the organism was grown. The peptone, as will be seen later, did not induce protease formation.

Hemicellulose was slightly hydrolyzed as is evidenced by the small reduction of Fehling's solution, and this may be taken to indicate cytases. The hemicellulose was prepared from date endosperm by the method of Zeller.[27] There was no cellulase activity exhibited. The test material in this case was filter paper cellulose prepared by the method of McBeth and Scales.[16] Further attention to the cyto-hydrolyzing enzymes is being given in connection with some histological and microchemical work now in progress.

Pectinase was tested for by comparing the ability of active and autoclaved powder to macerate living tissues. Disks of potato tuber, 400 mm. in thickness, were made by use of a cork borer and sliding microtome. The disks were placed in water extracts of the enzyme powder and the "cultures" preserved with toluol. At intervals disks were removed and their coherence tested. Scarcely any difference could be detected up to the 16th day of incubation, when the disks in the active extract tore slightly more easily than those in the autoclaved extract. Carrot and red beet disks were similarly tested; there was no apparent difference between the active enzyme extract and the check. The amount of red pigment of the beets that diffused into the extract of active enzyme was not greater than that with the autoclaved powder. The enzyme powder would not coagulate commercial pectin ("Certo").

Of the simple carbohydrates, raffinose and maltose were appreciably hydrolyzed. Sucrose was very strongly inverted. Of the three disaccharides lactose was least attacked.

In order to test the ability of the fungus to use these carbohydrates as sources of carbon they were added in quantities making 1 per cent of the total volume to synthetic media of the following formulae:

	Grams per liter		
MgSO₄ . 7 H₂O	0.50	0.50	.50
K₂HPO₄	1.00	1.00	1.00
KCl	.50	0.50	.50
NaNO₃	2.00	2.00 Ca(NO₃)₂	10.00
FeSO₄	.01	.01	.01
Agar	20.00	0.00	20.00

A comparison of the amount of extension of the mycelium in the carbohydrate cultures with that in the checks having no carbon other than that as agar, CO_2, impurities, and that stored in the mycelium indicated that all the carbohydrates could be used by the fungus, with the possible exceptions of lactose, inulin, and cellulose. The colonies in these media showed only slight increase in growth over those of the checks, and the cellulose medium showed no clearing zone near the fungus. It must be said, however, that such observations were unsatisfactory, owing to the poor growth of the organism on these synthetic media.

GLUCOSIDASES

Five glucosides were used to test for the respective glucosidases as shown in the following table. Here again the results represent net reduction after deduction for the checks:

TABLE 5

ACTION OF THE FUNGUS POWDER ON GLUCOSIDES

Substrate	Incubation period (*days*)	Temperature (°C.)	Reducing power (mgm. Cu liberated from Fehling's sol. by 1 ml. of substrate)
1. Hesperidin	2	29	1.8556
2. Phloridzin	3	37	5.285
3. Amygdalin	3	37	13.895
4. Salicin	3	37	16.6476
5. Arbutin	2	29	49.7404

The strikingly large hydrolysis of amygdalin, salicin and arbutin prove the presence of the B-glucosidase (emulsin) in the Pythiacystis. In the case of amygdalin a strong odor of benzaldehyde was soon very evident in the enzyme cultures and also in the fungus cultures having amygdalin as a source of carbon. A solution of arbutin in the presence of the enzyme powder or inoculum of the fungus soon acquired the

color of a quinole solution. Although, as stated by van Rijn,[23] phloridzin is not attacked by emulsin, there is evidence here that the Pythiacystis can slowly bring about its hydrolysis. Judging by the small amount of reduction obtained, the presence of a glucosidase capable of hydrolyzing hesperidin is doubtful. One per cent phloridzin and arbutin as carbon sources were incapable of supporting growth of Pythiacystis on synthetic agar media; salicin and amygdalin permitted a small extension of the mycelium; and hesperidin still a smaller amount. It should be reiterated, however, that the organism makes such a poor growth on synthetic media in general that these comparative observations are not satisfactory.

Although the fungus was capable of tolerating a concentration of 0.1 per cent tannin (Merck's digallic acid) in glucose-potato-peptone broth medium, and .05 per cent in prune broth and Czapek's synthetic medium, no evidence has yet been secured for the presence of tannase. The official Procter-Löwenthal method [see Official Methods[2] ('21), p. 274] was used to determine tannin, and Jean's[13] [see Zeller,[27] p. 507] iodometric method for gallic acid.

AMIDASES

The three amino acids, alanine, tyrosine and asparagin were used to test for deaminases. The asparagin, being also an acid amide, may be used to reveal the presence of deamidases. Acetamide and urea were the other substances employed, the former for deamidase and the latter for urease. The aeration method of Folin was used to estimate the ammonia formed.

TABLE 6

ACTION OF FUNGUS POWDER ON AMINO ACIDS AND ACID AMIDES

Substrate	Incubation period (days)	Temp. (°C.)	Initial (PH)	Final (PH)	Mgm. Ammonia N in 1 ml. of substrate. (Increase over check)
Alanine	4	37½			0.0
Alanine	23	37½		7.4	0.0
Tyrosine	4	37½			0.0
Acetamide	4	37		5.3	0.0
Acetamide	4	37		2.8	0.0323
Acetamide	4	37		5.2	0.0
Asparagin	2	29			0.0
Asparagin	5	37	9.0		1.067
Asparagin	5	37	2.4		0.0
Asparagin	23	37	8.6	8.7	.744
Urea	2	29			.865

Under the conditions employed no deaminase could be demonstrated and no deamidase with the acetamide. Asparagin gave a strongly positive test if the reaction was first adjusted to the alkaline side. Urease was demonstrated. The enzyme histozyme, which splits hippuric acid into glycine and benzoic acid, was tested for by determining the amino acid content of the substrate and by attempting to find benzoic acid after the incubation. The tests were negative, there being no increase in nitrogen liberated by nitrous acid, and no benzoic acid formed. In liquid media asparagin would serve as a source of N, and to a small extent as a source of both nitrogen and carbon. Urea would supply nitrogen, but not carbon and nitrogen in liquid media. The organism made no growth whatever on the agar media with urea present either with or without dextrose.

Acetamide appeared to be able to serve in a small measure as a source of nitrogen in liquid media, and very doubtfully as a source of both carbon and nitrogen. The tyrosine, alanine and hippuric acid were not tested in this way.

PROTEASES

In testing for proteoclastic enzymes an increase in aliphatic amino nitrogen, as determined in the van Slyke micro apparatus, was taken as positive evidence. Several other qualitative tests were employed, as will be explained.

TABLE 7

ACTION OF FUNGUS POWDER ON PROTEINS

Substrate	Incubation period (*days*)	Temp. (°C.)	Initial (Pн)	Final (Pн)	N₂ gas (ml. from 2 ml. of substrate; increase over that of checks)
Peptone	2	29			0.00
Gelatine	2	29			0.00
Leucosine	6	37½	9.0	6.1	0.00
Leucosine	6	37½	7.0	5.9	.0695
Leucosine	6	37½	2.6	3.7	0.00
Leucosine	7	38			.3295

The leucosine was made according to the method described by Onslow[16] (p. 25). The qualitative tests for tryptophane with bromine water and amyl alcohol were negative. With the possible exception of the leucosine none of the materials were hydrolyzed. In

the two instances of the slight hydrolysis of leucosine the substrate was about neutral in reaction. Both the enzyme powder and the fungus itself failed to liquify nutrient gelatines, although the organism made a vigorous growth on this medium. The acetondauerhefe powder and inoculum of *Fusarium lycopersici* tried at the same time caused a rapid liquefaction of the gelatine.

In another experiment pulverized blood fibrin was stained with a one-half of one per cent aqueous solution of congo red, and then thoroughly washed and dried (see Reed[18]). One gram quantities of this were placed in 200-ml. Erlenmeyer flasks having the enzyme powder, 20 ml. of water, and 1 ml. of toluol. To some of the flasks were added 1 ml. quantities of .1 N H_2SO_4 or .1 N NaOH. The cultures were incubated 4 days at 37½ degrees C; this was followed by 12 days at roőm temperature (17–25 degrees C). In no case was there appreciable evidence of the protein having been attacked, which in this test is indicated by a liberation of the dye into the liquid. In the cultures having NaOH there developed a slight pink in the water showing that some Congo red had been liberated, but an amino-nitrogen determination did not reveal any increase in NH_2 groups. From these results one must conclude that proteoclastic enzymes are absent or at most very feeble in the enzyme powder employed. This material also failed to coagulate milk.

Qualitative tests were made for zymase, peroxidase, oxidase, catalase, reductase, and tyrosinase by the procedure described by Onslow[16] (pp. 23, 24, 25 and 128) and the presence only of peroxidase and catalase demonstrated.

A quantitative test was made for glycolase, the enzyme which decomposes dextrose to form lactic acid. To 20 ml. of an approximately 1 per cent glucose solution were added 250 mgm. of enzyme powder and 1 ml. of toluol; this system was incubated four days at 37° C. Loss in power to reduce Fehling's solution was taken as an indication of the presence of the enzyme. This loss amounted to 1.1008 mgm. Cu per ml. of solution and was assumed to indicate a weak glycolase activity. The solution with the active enzyme showed also a slightly higher total acidity than the one with the autoclaved enzyme. Waksman and Davison[26] (p. 249) point out that toluene is injurious to this enzyme, which fact may account for the small amount the glucose destroyed.

DISCUSSION AND SUMMARY

In a survey of the enzymes of the mycelium of *Pythiacystis citro-phothora* the following were tested for: esterases, cellulase, cytase, pectinase (pectase), inulase, diastase, raffinase, invertase, lactase, maltase, emulsin (amygdalase, salicinase, arbutinase), glucosidases that attack hesperidin and phloridzin, tannase, amidases (deaminases, deamidases, urease) histozyme, proteases, rennet, zymase, peroxidase, oxidase, catalase, reductase, tyrosinase, and glycolase.

Very positive evidence was obtained for the presence of some of the lower esterases, for diastase, invertase, maltase, emulsin, phlorid-zinase, asparaginase, urease, peroxidase, and catalase; less evidence is forthcoming for the presence of cytase, lactase, hesperidinase; very slight indication of the presence of inulase, pectinase, protease and glycolase; and for the remaining enzymes sought the results were entirely negative.

The necessity for check determinations on both the active and deactivated enzyme material is emphasized, and a more accurate method of calculation given.

The diastase of this fungus attacks gelatinized starch vigorously, but starch suspended in cold water only feebly.

Although urea solution of the strength tried could not be used by the fungus, the enzyme powder gave a strong urease reaction.

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THE IMMUNIZATION OF FOWLS AGAINST CHICKEN-POX (EPITHELIOMA CONTAGIOSUM) BY SUBCUTANEOUS INJECTION OF VIRUS

J. R. BEACH[1]

INTRODUCTION[2]

The studies reported herein consist of a series of experiments in the immunization of fowls against chicken-pox (*Epithelioma contagiosum*) by the subcutaneous injection of vaccine containing the lesion tissue removed from fowls affected with the disease. The first report of a successful attempt to immunize fowls against chicken-pox by such means was that of Manteufel[1] in 1910. He reported success in immunizing fowls by injecting into the circulation or under the skin a lymph prepared from scraping of lesions on the comb or mucous membranes of the head of diseased birds mixed with physiologic salt solution and heated in a water bath at 55° C for one hour. According to this investigator, chickens treated in this manner were immune to infection for from one and one-half to two years even though there was no visible reaction following vaccination. Marked curative value for this preparation was also claimed.

Hadley and Beach[2] in 1913 and Mack and Records[3, 4] in 1915 and 1916 reported the results of experiments with vaccine prepared after the method of Manteufel. They concluded that it was highly effective in the preventing of chicken-pox and exerted a curative effect on diseased fowls. No standard for the preparation of vaccine was adopted by any of the previously mentioned investigators.

The writer[5] in 1920 reported the results of the experimental use of vaccine prepared according to a modification of the Manteufel method. In this vaccine only the scabs from the chicken-pox lesions on the comb were used. This material was secured by artificial propagation on healthy cockrels. After removal, the scabs were dried and reduced to a fine powder. A standardized method of preparation was adopted. This consisted of mixing one gram of the dessicated virus with 100 cubic centimeters of sterile physiologic saline. The mixture was heated in a water bath at 55° C for one hour and preserved by the addition of 0.2 per cent of tricresol. This vaccine was reported to confer either immunity or increased resistance against infection with chicken-pox virus. Curative properties were also claimed for it. The immunity or resistance to infection after vaccination was not determined to be lasting, and, therefore, the vaccine was recommended for use in the control of outbreaks of chicken-pox in infected flocks rather than as a means of protecting healthy flocks against subsequent infection.

Experiments in the control of outbreaks of chicken-pox by Boerner and Stubbs,[6] reported in 1921, failed to confirm these results. These investigators concluded that vaccine prepared according to either method was not demonstrated to be of any value.

Quite different conclusions regarding the value of dry-virus vaccine, however, were reported by Fuller[7] in 1924 and Gwatkin[8] in 1925. Both of these investigators stated that their experiments demonstrated that the vaccine was of considerable value in controlling outbreaks of chicken-pox.

Vaccine prepared from dried virus has been very extensively used on poultry flocks in California in recent years. In the majority of cases satisfactory results have been obtained but not infrequently no apparent benefit was derived from vaccination. Such variation in results of the practical application of this method of vaccination on a large scale is in agreement with the results of the previously described experimental vaccination. The need for an improved method of preparation of vaccine is, therefore, apparent. It was for this purpose that the experiments described in the following pages were undertaken.

METHODS EMPLOYED

Certain of the methods of procedure were the same in all experiments. In order to avoid a repetition in the discussion of each experiment the following description of these methods is given at this point:

Material for the Preparation of Vaccine.—Material was secured from cockerels that had been inoculated with chicken-pox virus. The method of inoculation consisted in thoroughly scarifying the skin of both sides of the comb and applying a suspension of highly virulent chicken-pox virus to the scarified surface. Cockerels with the scarified area evenly covered with pronounced lesions were selected for use.

Virulence Tests.—Tests were made of all vaccines for the purpose of determining the presence of living virulent chicken-pox virus in the vaccines. They consisted of scarifying about 1 sq. cm. of the comb surface or of making four or five deep scratches in the skin of the comb with a large, dull hypodermic needle or a small trephine that had been dipped in the vaccine.

Immunizing Tests.—These tests consisted in vaccinating cockerels and later inoculating them with virulent chicken-pox virus. The method of vaccination was subcutaneous injection in the breast under the right wing with a 16 to 18-gauge hypodermic needle. The inoculation of the vaccinated birds was performed in the same manner as the virulence test except that the needle or trephine was dipped in a suspension of virulent virus instead of vaccine. Non-vaccinated, control cockerels, inoculated in the same manner with the same virus as the vaccinated birds, were included in each immunizing test. In all cases the virus used was found to be highly virulent.

In order that all of the experimental birds could with reasonable certainty be regarded as susceptible to chicken-pox, White Leghorn cockerels from ten to fourteen weeks old were used exclusively.

EXPERIMENTS WITH VACCINE PREPARED FROM WHOLE COMBS

This type of vaccine was prepared from the combs of cockerels that had been killed from nine to twelve days after inoculation with chicken-pox virus. Cockerels with nearly the entire comb surface evenly covered with chicken-pox lesions were selected. Three lots of

vaccine were prepared from such material. There were certain differences in the methods of preparing the vaccines as indicated in the following descriptions:

Vaccine No. 1.—Vaccine No. 1 was prepared on August 6, 1924, from the combs removed from two cockerels on the ninth day after inoculation with chicken-pox virus. The combs were cut into small pieces and triturated in a mortar with sterile sand and a small amount of 0.5-per-cent phenolized physiologic salt solution for more than three hours. Sufficient phenolized salt solution to provide 10 cc. for each gram of tissue was then added and the mixture filtered through gauze and filter paper. The resulting filtrate was a yellowish turbid liquid.

Vaccine No. 2.—Vaccine No. 2 was prepared on September 22, 1924, in the same manner as vaccine No. 1, from the combs removed from cockerels on the tenth day after inoculation with chicken-pox virus.

Vaccine No. 3.—Vaccine No. 3 was prepared March 24, 1925, from combs removed from cockerels on the tenth day after inoculation with chicken-pox virus. The diluent of this and later vaccines was a mixture of equal parts of glycerine and 1.0-per-cent phenolized physiologic salt solution. In a series of tests it was found that chicken-pox virus may remain alive in such a mixture for more than two years. It was thought therefore that it might be more desirable for use in the preparation of vaccine than the phenolized physiologic saline. The tissue was first put through a small Enterprise mill[3] (fig. 1) and then, with the addition of a small amount of diluent, through a special tissue mill (fig. 3) in which it was reduced to an extremely fine state. The mixture was next strained through a fine wire screen to remove the shreds of fibrous tissue that were not ground fine in the tissue mill. Sufficient of the glycerine-phenolized-saline mixture was then added to provide 10 cc. for each gram of tissue recovered from the Enterprise mill.

The results of the immunizing and virulence tests of the three vaccines appear in table 1.

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[3] The Enterprise mill was later replaced by a Latapie grinder (fig. 2) which more satisfactorily prepared the tissue for the special tissue mill.
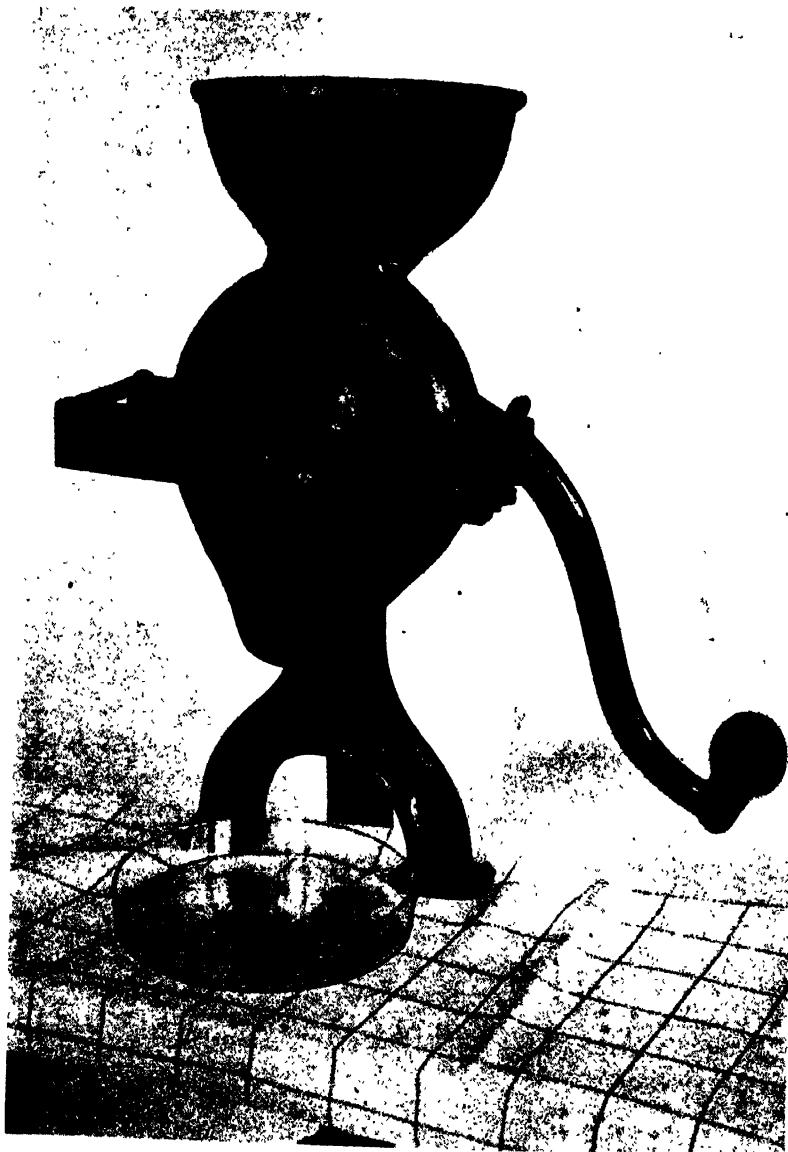
Fig. 1.   Enterprise mill, No. 0, remodelled to permit removal of all grinding parts for cleaning and sterilizing. This mill is designed for grinding dry material and is not entirely satisfactory for fresh tissue.
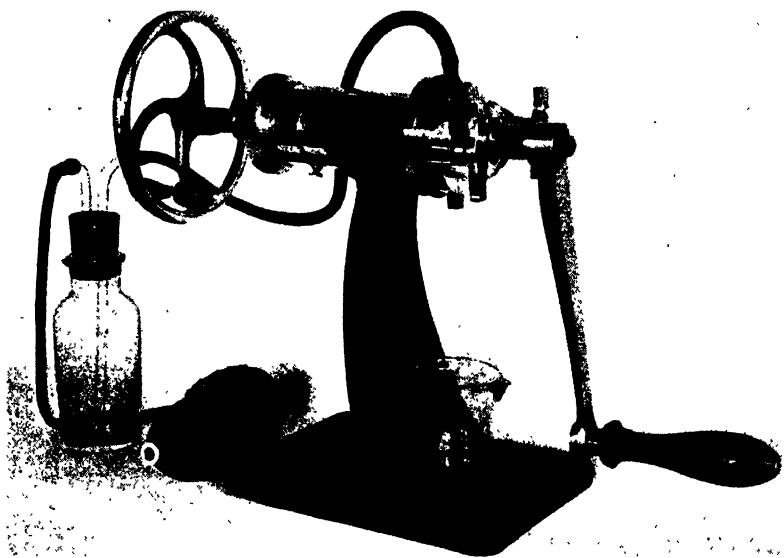
Fig. 2. Latapie grinder, designed especially for grinding fresh tissue. Procured from Cogit et Cie., Boulevard Saint-Michel, 36, Paris, France.
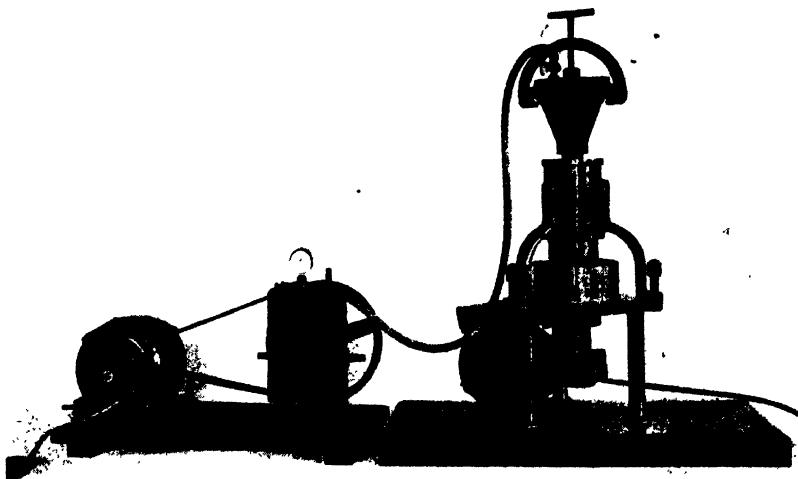
Fig. 3. A substantially constructed mill for fresh tissue, modelled after one used by the Cutter Laboratories, Berkeley, California, in the preparation of smallpox vaccine. Air pressure is used to hasten the passage of material through this mill.

TABLE 1

RESULTS OF IMMUNIZING AND VIRULENCE TESTS OF WHOLE-COMB VACCINES 1, 2, AND 3

Vaccine No.	Test No.	Vaccine Age (days)	Vaccine Number of doses	Vaccine Am't of each dose cc	Vaccine Number vaccinated	Number that developed chicken-pox lesions at vaccination point	Number that developed chicken-pox lesions on head	Number tested for immunity and days after vaccination when tested	Number immune or that developed chicken-pox	Number of controls inoculated	Lesions produced	Test No.	Age of vaccine (days)	Number inoculated	Lesions produced
1	1	1	1	1	4	...	0	2 in 14 days	1 slight lesions / 1 marked lesions	2ᵃ	Marked lesions on both.				
								2 in 28 days	Both immune	2ᵇ	Moderate lesions on one / Marked lesions on one				
1	1	1	1	5	4	...	0	2 in 14 days	Both immune	2ᵃ	Marked lesions on both				
								1ᶜ in 28 days	Both immune	2ᵇ	Moderate lesions on one / Marked lesions on one				
1	1	1	1	10	4	...	0	2 in 14 days	Both immune	2ᵃ	Marked lesions on both / Moderate lesions on one				
								1ᶜ in 28 days	Bird immune	2ᵇ	Marked lesions on one				
1	2	25	2	2	10	...	0	10 in 14 days	7 immune / 3 slight lesions	9	Marked lesions on all	No virulence tests of vaccine No. 1 were made.			
2	1	1	1	2	8	...	4	4 in 14 days / 4 in 28 days	4 moderate lesions / 4 immune	5ᵈ / 4ᵉ	Marked lesions on all / Marked lesions on all				
2	1	1	2	2	8	...	3	2 in 14 days / 4 in 28 days	2ᶠ slight lesions / 4 immune	5ᵈ / 4ᵉ	Marked lesions on all / Marked lesions on all	No virulence tests of vaccine No. 2 at this age were made			

ᵃ Same controls for all groups inoculated on the same day.
ᵇ Same controls for all groups inoculated on the same day.
ᶜ One bird died from causes not related to vaccination or chicken-pox.
ᵈ The same 5 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 14 days after vaccination.
ᵉ The same 4 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 28 days after vaccination.
ᶠ Two birds died from causes not related to vaccination or chicken-pox.
ᵍ Three birds died from causes not related to vaccination or chicken-pox.
ʰ These birds had not developed vaccine-injection-point lesions.

		Vaccine						Immunising tests				Virulence tests			
Vaccine No.	Test No.	Age *days*	Number of doses	Am't of each dose *cc*	Number vaccinated	Number that developed chicken-pox lesions at vaccination point	Number that developed chicken-pox lesions on head	Number tested for immunity and days after vaccination when tested	Number immune or that developed chicken-pox	Number of controls inoculated	Lesions produced	Test No.	Age of vaccine *days*	Number inoculated	Lesions produced
2	1	1	2	1	8	...	2	4 in 14 days	3 immune; 1 slight lesions	5d	Marked lesions on all	No virulence test of vaccine No. 2 at this age were made.			
								3c in 28 days	3 immune	4e	Marked lesions on all				
2	1	1	2	0.5	8	...	0	3c in 14 days	1 immune; 2 slight lesions	5d	Marked lesions on all				
								2g in 28 days	2 immune	4e	Marked lesions on all				
2	2	29	2	0.5	20	...	0	20 in 14 days	20 slight lesions	19	Marked lesions on all	1	32	2	Marked lesions on both.
2	3	57	2	0.5	40	...	0	18f in 14 days	18 marked lesions; 4 immune	20	Marked lesions on all	2	51	2	Marked lesions on both.
								17h in 28 days	13 slight to marked lesions	18	Marked lesions on all				
3	1	1	2	0.5	5	3	0	4e in 28 days	4 very slight lesions	5	Marked lesions on all				
		1	2	1	5	3	0	4e in 28 days	4 immune						
		1	2	2	5	3	0	4e in 28 days	4 immune						
3	2	...	1	0.5	3	3	0	2c in 28 days	2 immune	6	Marked lesions on all	1	25	2	Marked lesions on both.
		...	2	0.5	3	3	0	3 in 28 days	3 immune						
		25	1	1	3	3	0	3 in 28 days	3 immune						
		...	2	2	3	3	0	3 in 28 days	3 immune						
		...	2	2	3	3	0	3 in 28 days	3 immune						

a Same controls for all groups inoculated on the same day.
b Same controls for all groups inoculated on the same day.
c One bird died from causes not related to vaccination or chicken-pox.
d The same 5 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 14 days after vaccination.

e The same 4 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 28 days after vaccination.
f Two birds died from causes not related to vaccination or chicken-pox.
g Three birds died from causes not related to vaccination or chicken-pox.
h These birds had not developed vaccine-injection-point lesions.

TABLE 1—(Continued)

Vaccine No.	Test No.	Vaccine Age days	Vaccine Number of doses	Vaccine Am't of each dose cc	Number vaccinated	Number that developed chicken-pox lesions at vaccination point	Number that developed chicken-pox lesions on head	Number tested for immunity and days after vaccination when tested	Number immune or that developed chicken-pox	Number of controls inoculated	Lesions produced	Test No.	Age of vaccine days[a]	Number inoculated	Lesions produced
3	3	....	1	0.5	3	3	0	3 in 28 days	3 immune	6	Marked lesions on all	2	45	2	Marked lesions on both.
		....	2	0.5	3	3	0	3 in 28 days	3 immune						
		45	1	1	3	3	0	2c in 28 days	3 immune						
		....	2	1	3	3	0	3 in 28 days	2 immune						
		....	1	2	3	3	0	3 in 28 days	3 immune						
		....	2	2	3	3	0	3 in 28 days	3 immune						
3	4	....	1	0.1	3	2c	0	2c in 28 days	2 immune	6	Marked lesions on all	3	86	2	Marked lesions on both.
		....	2	0.1	3	0	0	3 in 28 days	3 immune						
		86	1	0.5	3	2	0	3 in 28 days	3 immune						
		....	2	0.5	3	2	0	3 in 28 days	3 immune						
		....	1	1	3	3	0	3 in 28 days	1 immune / 2h moderate lesions						
		....	2	1	3	0	0	3 in 28 days	3 immune						
		86	1	2	3	3	0	3 in 28 days	2 immune						
		86	2	2	3	2	0	3 in 28 days	1b moderate lesions						
3	5	....	1	0.5	3	0	0	3 in 28 days	All marked lesions	6	Marked lesions on all	4	183	2	None.
		....	2	0.5	3	0	0	3 in 28 days	All marked lesions						
		183	1	1	3	0	0	3 in 28 days	All marked lesions						
		....	2	1	3	0	0	3 in 28 days	All marked lesions						
		....	1	2	3	0	0	3 in 28 days	All marked lesions						
		....	2	2	3	0	0	3 in 28 days	All marked lesions						

a Same controls for all groups inoculated on the same day.
b Same controls for all groups inoculated on the same day.
c One bird died from causes not related to vaccination or chicken-pox.
d One bird died from causes not related to vaccination or chicken-pox.
e The same 5 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 14 days after vaccination.
f The same 4 birds served as controls for all birds vaccinated with 1-day-old vaccine No. 2 and inoculated 28 days after vaccination.
g Two birds died from causes not related to vaccination or chicken-pox.
h Three birds died from causes not related to vaccination or chicken-pox.
i These birds had not developed vaccine-injection-point lesions.

Discussion of Results.—Eight of the twelve cockerels that received a subcutaneous injection of 1 cc., 5 cc., or 10 cc. of fresh vaccine No. 1 were immune to artificial infection with chicken-pox virus 14 or 28 days later. Two of the cockerels that were inoculated 14 days after vaccination with a 1-cc. dose of vaccine developed chicken-pox lesions which, however, were less marked than those which developed on the controls. Two of the cockerels were lost to the experiment from causes not related to vaccination. Seven of ten cockerels vaccinated with two 2-cc. doses of the vaccine, 25 days after preparation were found to be immune to chicken-pox when they were inoculated 14 days after vaccination. Slight chicken-pox lesions developed in the remaining three cockerels. No harmful effect or reaction of any nature was observed in any of the birds after vaccination.

Nine of the twenty-four birds that were vaccinated with one or two 2-cc. doses or two 1-cc. doses of the fresh vaccine No. 2 developed one or two small chicken-pox tumors on the comb or wattles in from 12 to 18 days after vaccination. These lesions did not spread and disappeared in about 10 days. Ten of these twenty-four birds were inoculated 14 days after vaccination. Three of the ten were immune. Seven developed slight or moderate chicken-pox lesions. Eleven of the twenty-four birds were inoculated in 28 days after vaccination. All were immune. Three birds died before the time for inoculation arrived. None of the eight birds that received two 0.5-cc. doses of fresh vaccine became infected from the vaccine. Inoculation with chicken-pox virus on the fourteenth day after vaccination with three birds and the twenty-eighth day after vaccination with two birds, however, showed them to be just as resistant to the infection as the birds that had received larger doses of vaccine. This made it appear that such a dosage might be more satisfactory than a larger one. Consequently twenty cockerels were given two 0.5-cc. doses with 20-day-old vaccine. They were inoculated 14 days after vaccination. All were highly resistant to the infection but none were entirely immune. Another lot of forty birds were vaccinated with the same dose when the vaccine was 57 days old. None became infected from the vaccine. Five were lost from the experiment by death from causes unrelated to vaccination. Eighteen were inoculated on the fourteenth day after vaccination. All of these developed marked chicken-pox lesions. Seventeen were inoculated on the twenty-eighth day after vaccination. Four were immune. The others developed slight to marked lesions. These results indicate that the vaccine was decreasing in immunological value as it increased in age. It is presumed that the immunological value is to a large degree dependent upon the amount and

virulence of the virus present. Tests failed to demonstrate that the virulence in the 57-day-old vaccine was less than in that 32 days old. Experience has shown, however, that fowl inoculation would detect only marked differences in virulence.

In the experiments with vaccine No. 3, all inoculations of vaccinated birds with chicken-pox virus were made on the twenty-eighth day after vaccination, since the results with vaccine No. 2 had indicated that this was a more suitable time than the fourteenth day.

Comparative tests of one and two doses of 0.1 cc., 0.5 cc., 1 cc., and 2 cc. of vaccine and of 1-day, 25, 45, 86, and 183-day old vaccine were performed on a total of ninety-three birds.

No chicken-pox lesions appeared about the head of any bird as a result of the injection of vaccine. In the course of the examinations of the first lot of birds vaccinated, however, a dry scab resembling a chicken-pox lesion was observed on the skin of some birds at the point of vaccination. The scab was removed, ground in a mortar with sterile saline and applied to a scarified surface of the skin of two normal cockerels. Marked chicken-pox lesions were produced, thus definitely establishing that the lesions observed were chicken-pox. This observation was made too late to permit the securing of data on the occurrence of such lesions on this lot of birds. Such data were secured, however, from all birds that were vaccinated later.

Vaccine-injection-point chicken-pox lesions appeared on all but nine of the sixty cockerels that were vaccinated with the 25, 45, or 86-day-old vaccine. The exceptions were all among those that received the 86-day-old vaccine. Three of the nine received two 0.1-cc. doses, one received one 0.5-cc. dose, one received two 0.5-cc. doses, three received two 1-cc. doses, and one received one 2-cc. dose. It is seen, therefore, that no relationship existed between the size of the dose of vaccine and the development of vaccine-injection-point lesions. In those birds that received two doses of vaccine the lesions appeared only at the point of the first injection. They reached sufficient size to be definitely recognized in from 7 to 10 days and persisted for from 10 to 20 days. In all cases the lesions had healed when the fowls were inoculated with chicken-pox virus on the twenty-eighth day after vaccination. In most instances the vaccine-injection-point lesion consisted of a single tumor from 2 mm. to 4 mm. in diameter. On a few birds, however, the lesions covered an area as large as from 10 mm. to 15 mm. in diameter. In no case did the infection become general or in any way harm the fowls.

Six birds were lost from the experiment by death. The immunity test was completed with eighty-seven birds.

Of sixty-nine birds in the test of 1, 25, 45, or 86-day-old vaccine, sixty-two were immune and seven developed slight chicken-pox lesions after inoculation on the twenty-eighth day after vaccination. Of the seven birds that were slightly susceptible to the infection, four had received two 0.5-cc. doses of 1-day-old vaccine and two had received two 1-cc. doses and one had received two 2-cc. doses of 86-day-old vaccine. The failure of these birds to become entirely immunized cannot be ascribed either to the size or number of the doses or to the age of the vaccine, since other birds were immunized by a smaller dose of vaccine of the same age or older.

Three of the non-immune birds were from those that did not develop vaccine-injection-point lesions. This cannot be considered the reason for their remaining slightly susceptible to chicken-pox, however, because six other birds that had had no vaccination lesions were found to be immune.

The data concerning the sixty-eight birds that were immunized by vaccination show that doses of 0.1 cc., 0.5 cc., 1 cc., and 2 cc. were equally effective, that one dose of any of these amounts was as effective as two doses, and that no apparent decrease in the effectiveness of the vaccine had occurred by keeping it for 86 days. The virulence tests showed no discernable difference in the virulence of the virus contained in the 25, 45, and 86-day-old vaccine. The 183-day-old vaccine failed to produce any vaccination lesions, to immunize or increase the resistance to chicken-pox of any of the eighteen birds that were vaccinated with it, or to produce any lesions on the fowls used in the virulence test.

Vaccine No. 3 after 86 days storage proved to be a more potent immunizing agent than vaccine No. 2 after 57 days storage. The only essential difference between the two vaccines was that the glycerine-phenolized-saline mixture was used in the preparation of the former and phenolized saline alone in the latter. This suggests that the mixture of equal parts of glycerine and 1.0-per-cent phenolized salt solution is more suitable for use than the phenolized saline alone.

The results of this series of experiments show that fowls can be immunized against artificial infection with chicken-pox virus in 28 days by subcutaneous injection with vaccine prepared from fresh comb and lesion tissue of cockerels with marked chicken-pox infection.

Evidence was also procured to show that, although the vaccine contains virulent chicken-pox virus and its injection is liable to be attended by the development of slight chicken-pox lesions on the skin at the point of injection, it is not liable to cause infection at other locations, such as about the head.

EXPERIMENTS WITH VACCINES PREPARED FROM WHOLE COMBS, BLOOD, AND ORGANS

The purpose of the experiments with vaccines Nos. 4, 5, and 6 was to determine if the inclusion of the blood, liver, spleen, and kidneys of cockerels with marked chicken-pox infection with the comb and lesion tissue would result in a vaccine of greater immunizing value than one containing comb and lesion tissue only.

Vaccine No. 4.—The first lot of this type of vaccine, No. 4, was prepared on March 24, 1925. A cockerel was killed in a gas chamber on the tenth day after inoculation with chicken-pox virus. The abdominal and thoracic cavities were immediatley opened, the aorta severed and the blood allowed to collect in the abdominal cavity. The blood, liver, spleen, kidneys, and comb were removed for the preparation of the vaccine. The method of preparation was the same as that of vaccine No. 3. The proportion of diluent to tissue was 10 cc. per gram. The weight of the blood and organs was 20 grams and of the comb and lesion tissue 16 grams. Each cubic cent'meter of this vaccine, therefore, contained less than half the amount of the comb and lesion tissue in a like amount of vaccine No. 3.

Immunizing and virulence tests were made with fresh vaccine and vaccine 25, 45, 86, and 183 days old. In all cases the cockerels used in the immunizing tests were inoculated with chicken-pox virus on the twenty-eighth day after vaccination.

The results of the immunizing and virulence tests are summarized in table 2.

As shown in table 2, all of the sixty-nine cockerels that were given one or two subcutaneous injections of 0.1 cc., 0.5 cc., 1 cc., or 2 cc., each of vaccine No. 4, when it was 1, 25, 45, or 86 days old, were immune to artificial infection with chicken-pox virus 28 days later. The eighteen birds that were vaccinated with one or two 0.5-cc., 1-cc., or 2-cc. doses of 183-day-old vaccine exhibited neither immunity nor resistance to artificial infection 28 days later. The same results were obtained with the smallest dose and the largest dose of vaccine, and with one dose and two doses.

The results of the virulence tests showed that the 1, 25, 45, and 86-day-old vaccine contained virulent chicken-pox virus and did not indicate that the virulence had decreased with age. No virulence was demonstrated in the 183-day-old vaccine.

TABLE 2

Results of Immunizing and Virulence Tests of Vaccine No. 4

Immunising tests

Test No.	Vaccine Age (days)	Vaccine Number of doses	Vaccine Amount of each dose cc	Number vaccinated	Number that developed vaccination-point lesions[a]	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced
1	1	2	0.5	5	.....	3[b]	3 immune	5	Marked lesions on all
		2	1	5	.....	3[b]	3 immune		
		2	2	5	.....	5	5 immune		
2	25	1	0.5	3	3	3	3 immune	6	Marked lesions on all
		2	0.5	3	3	3	3 immune		
		1	1	3	3	2[c]	2 immune		
		1	2	3	3	3	3 immune		
		2	2	3	3	3	3 immune		
3	45	1	0.5	3	3	3	3 immune	6	Marked lesions on all
		2	0.5	3	3	3	3 immune		
		1	1	3	3	3	3 immune		
		2	1	3	3	2[c]	2 immune		
		1	2	3	3	3	3 immune		
		2	2	3	3	3	3 immune		

Virulence tests

Test No.	Age of vaccine (days)	Number inoculated	Lesions produced
1	1	2	Marked
2	25	2	Marked
3	45	2	Marked

[a] No examination for vaccination-point lesions were made at the first test.
[b] Two died from causes not related to vaccination.
[c] One died from causes not related to vaccination.

TABLE 2—(Continued)

Test No.	Vaccine Age days	Vaccine Number of doses	Vaccine Amount of each dose cc	Number vaccinated	Number that developed vaccination-point lesions[a]	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Virulence Test No.	Age of vaccine days	Number inoculated	Lesions produced
4	86	1	0.1	3	2	3	3 immune	6	Marked lesions on all	4	86	2	Marked
		2	0.1	3	1	3	3 immune						
		1	0.5	3	3	3	3 immune						
		2	0.5	3	0	3	3 immune						
		1	1	3	1	3	3 immune						
		2	1	3	1	3	3 immune						
		1	2	3	0	3	3 immune						
		2	2	3	0	3	3 immune						
5	183	1	0.5	3	0	3	3 marked lesions	6	Marked lesions on all	5	183	2	None
		2	0.5	3	0	3	3 marked lesions						
		1	1	3	0	3	3 marked lesions						
		2	1	3	0	3	3 marked lesions						
		1	2	3	0	3	3 marked lesions						
		2	2	3	0	3	3 marked lesions						

[a] No examination for vaccination-point lesions were made at the first test.
[b] Two died from causes not related to vaccination.
[c] One died from causes not related to vaccination.

Chicken-pox lesions at the point of injection of vaccine developed on all of the thirty-six cockerels that were vaccinated with the 25 and 45-day-old vaccines and on nine of the twenty-four birds that received the 86-day-old vaccine. These lesions could be definitely identified from 7 to 12 days after vaccination. They were entirely healed by from 20 to 28 days after vaccination. No lesions developed elsewhere on any of the birds. All of the seventeen birds vaccinated with the 86-day-old vaccine that did not develop lesions at the point where the vaccine was injected were immune to infection with chicken-pox virus 28 days after vaccination. This is additional evidence that the development of vaccine-injection-point lesions is not essential to the production of immunity. There were no vaccination-point lesions on the birds vaccinated with 183-day-old vaccine.

Vaccine No. 4 was prepared on the same date and tested in the same manner and on the same dates as vaccine No. 3. The immunizing tests of vaccine No. 4 yielded slightly better results than the corresponding tests with vaccine No. 3, although the former contained less than half as much comb and lesion tissue as the latter. The difference was not sufficiently marked, however, to warrant the conclusion that a vaccine prepared from the blood, liver, spleen, kidneys, comb, and lesions of a cockerel with marked chicken-pox infection of the comb is superior to one prepared from the comb and lesion tissue only as an agent for immunizing to chicken-pox infection. Virulence tests of the two vaccines gave identical results.

Vaccines 5 and 6.—For further comparison of the immunizing value of a vaccine containing the blood, organs, and comb and lesion tissue with one containing comb and lesion tissue only, two vaccines, Nos. 5 and 6, were prepared. The methods of preparation were the same as used for vaccines Nos. 3 and 4. Vaccine No. 5 contained the comb and lesion tissue and vaccine No. 6 the comb and lesions, blood, liver, spleen, and kidneys of cockerels that were killed on the ninth day after inoculation with chicken-pox virus. In each vaccine the proportion of the diluent to the tissue was 10 cc. to 1 gram. Each cubic centimeter of vaccine No. 5, therefore, contained more of the comb and lesion tissue than vaccine No. 6. In the immunizing and virulence tests this difference in the two vaccines was overcome by diluting them sufficiently with the glycerine-phenolized-saline mixture to make 1 cc. of each contain the same amount of comb and lesion tissue.

The results of the tests of vaccines Nos. 5 and 6 are given in table 3.

TABLE 3

RESULTS OF IMMUNIZING AND VIRULENCE TESTS OF VACCINES 5 AND 6*

Vaccine			Immunizing tests						Virulence tests	
No.	Type of tissue	Amount of comb tissue in dose[b] *grams*	Number of fowls vaccinated	Number that developed vaccination-point lesions	Number tested for immunity 28 days after vaccination	Number immune	Number of controls inoculated	Lesions produced	Number inoculated with vaccine	Lesions produced
5	Comb and lesion	0.063	3	3	2c	2			2	Marked
		.0093	3	2	3	3	3	Marked	2	Marked
		.00063	3	1	3	3			2	1 tumor on each bird.
6	Comb and lesions, blood, and organs	.0093	3	3	2c	2	3	Marked	2	Marked
		0.00063	3	3	3	3			2	None

a Vaccines were 4 days old when tests were made.
b Size of dose was uniformly 1 cc.
c One died from causes not related to vaccination.

As shown in table 3, all of the thirteen cockerels that were inoculated with chicken-pox virus in 28 days after vaccination were immune to the infection, irrespective of the type or amount of tissue in the vaccine.

Lesions developed at the point of injection of vaccine on six of the nine birds that received vaccine No. 5 and on all of six birds that received vaccine No. 6. The birds that did not develop vaccination lesions were one of three that received 0.0093 gram of comb tissue and two of three that received 0.00093 gram of comb tissue. Failure of vaccination lesions to appear, therefore, was not confined to those birds that received the smallest amount of comb and lesion tissue. These three birds were immune to infection with chicken-pox virus 28 days after vaccination, thus adding to the data showing that the development of lesions at the vaccination point is not essential to the development of immunity.

The virulence test demonstrated the presence of highly virulent virus in all dilutions of vaccine. The fact that but one tumor was produced on each of two birds and no lesions on two others that were inoculated with the dilutions containing 0.00093 gram of comb tissue per cubic centimeter, however, indicates that there was only a small amount of virus in that dilution.

The results of this experiment have failed to demonstrate any difference in the immunizing value of the two vaccines, both of which contained like amounts of comb and lesion tissue and one of which contained, in addition, the blood, liver, spleen, and kidneys of cockerels with marked chicken-pox infection.

EXPERIMENTS WITH VACCINES PREPARED FROM THE LESIONS OF THE COMB AND FROM THE BLOOD AND ORGANS

In the preceding comparative experiments it was found that vaccines prepared from the whole comb or from the whole comb, blood, liver, spleen, and kidneys of cockerels with marked chicken-pox lesions appeared to be equally effective in immunizing fowls against artificial infection with chicken-pox. It was not determined, however, whether the inclusion of the blood and organs added to the immunizing properties of vaccine. To obtain information on this point it therefore appeared necessary to prepare vaccines from such tissue alone and to make comparisons of the immunizing properties of such vaccines with others prepared from comb and lesion tissues.

For this purpose a series of five vaccines, Nos. 7, 8, 9, 10, and 11, were made from the blood, livers, spleens, and kidneys of the birds killed on the tenth, eleventh, twelfth, thirteenth, and fourteenth days, respectively, after inoculation and five other vaccines, Nos. 12, 13, 14, 15, and 16, from the lesions of the combs of the same birds. Birds were selected that had a uniform, heavy growth of chicken-pox lesions covering the surface of both sides of the comb, but that did not exhibit general symptoms of sickness, such as marked droopiness and inappetence.

The method of preparing the vaccines from the blood and organs was essentially the same as that of previous preparations. Sufficient diluent was added to make each cubic centimeter of vaccine contain 0.33 gram of tissue.

For the preparation of the vaccines from comb tissue, instead of the whole comb, the lesions and sublesion epithelial tissue only were used. This tissue was removed with a sharp scalpel, care being taken to include as little as possible of the subcutaneous fibrous tissue. The reasons for making this change were that the tough fibrous portions of the comb had proved very difficult to reduce to a fine state, that much of it had been removed when the vaccine was strained, and that the portion of it remaining in the vaccine was probably inert. The method of preparing the vaccines was otherwise the same as that previously described. The amount of the lesion tissue that was not ground finely enough to pass through the screen was negligible. Sufficient diluent was added so that 1 cc. of vaccine contained 0.1 gram of tissue.

In addition to determining the immunizing value of the vaccines these experiments were designed to furnish information regarding the presence of chicken-pox virus in the blood and internal organs of infected fowls; the minimum amount of lesion tissue which, when injected subcutaneously, will produce immunity to chicken-pox; and the most suitable time after inoculation to secure material for the preparation of vaccine from lesion tissue.

Some information on the latter question was furnished by the yield of lesion tissue by the birds killed on the different days after inoculation. The birds and the area of comb surface occupied by lesions were approximately the same size. The amount of tissue obtained was 9, 13, 19, 16, and 15 grams from the bird killed on the tenth, eleventh, twelfth, thirteenth, and fourteenth day, respectively, after inoculation. The increase in the amount of tissue from the tenth to the twelfth day was due to increase in the thickness of the lesion

TABLE 4

RESULTS OF FIRST IMMUNIZATION AND VIRULENCE TESTS OF VACCINES 7 TO 16. (Fresh vaccine used)

No.	Vaccine — Prepared from	Amount of tissue in dose (grams)	Number vaccinated	Number developed vaccination-point lesions	Number developed lesions on the head	Number tested for immunity 28 days after vaccination	Number immune or developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Number inoculated	Lesions produced	Incubation period (days)
7	Blood and organs from cockerel 10 days after inoculation	0.33	1	0	1ᵃ	1	1 immuneᵇ	2	Marked	1	Marked	10
		.66	1	0	0	1	Lesions producedᶜ					
8	Blood and organs from cockerel 11 days after inoculation	.33	1	0	0	1	1 immune	2	Marked	1	A few tumors	9ᵈ
		.66	1	0	0	1	Lesions produced					
9	Blood and organs from cockerel 12 days after inoculation	.33	1	1	0	1	1 immune	2	Marked	1	Marked	8
		.66	1	0	0	1	1 immune					
10	Blood and organs from cockerel 13 days after inoculation	.33	1	0	0	1	1 immune	2	Marked	1	Marked	8
		.66	1	1ᵉ	0	1	1 immune					
11	Blood and organs from cockerel 14 days after inoculation	.33	1	0	0	1	1 immune	2	Marked	1	Marked	15ᵈ
		.66	1	0	0	1	1 immune					

ᵃ Lesions probably not produced by the vaccine.

ᵇ Immunity probably due to comb infection rather than to the vaccine.

ᶜ Bird died 10 days after inoculation. Lesions active at time of death.

ᵈ Lesions were present at the same time on the opposite side of the comb. All lesions may therefore be due to accidental infection. If the lesions on the opposite side of the comb had resulted from inoculation, they would not have appeared until after the lesion at the point of inoculation.

ᵉ Lesions did not develop until the 24th day after vaccination. May not have been caused by the vaccine.

ᶠ These lesions not observed until the 28th day after vaccination. May have resulted from accidental infection rather than vaccination.

TABLE 4—(Continued)

Vaccine			Immunizing tests							Virulence tests		
No.	Prepared from	Amount of tissue in dose *grams*	Number vaccinated	Number developed vaccination-point lesions	Number developed lesions in the head	Number tested for immunity 28 days after vaccination	Number immune or developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Number inoculated	Lesions produced	Incubation period *days*
12	Lesions from cockerel 10 days after inoculation	.1 .03	2 2	2 2	0 1f	2 2	2 immune 2 immune	2	Marked	1 1	Marked Marked	6 6
13	Lesions from cockerel 11 days after inoculation	.1 .01	2 2	2 2	0 0	2 2	2 immune 2 immune	2	Marked	1 1	Marked Marked	6 6
14	Lesions from cockerel 12 days after inoculation	.1 .005	2 2	2 2	0 1f	2 2	2 immune 2 immune	2	Marked	1 1	Marked Marked	6 6
15	Lesions from cockerel 13 days after inoculation	.1 .002	2 2	2 2	0 0	2 2	2 immune 2 immune	2	Marked	1 1	Marked Marked	6 6
16	Lesions from cockerel 14 days after inoculation	.1 0 00:	2 2	2 2	0 0	2 2	2 immune 2 immune	2	Marked	1 1	Marked Marked	6 6

a Lesions probably not produced by the vaccine.
b Immunity probably due to comb infection rather than to the vaccine.
c Bird died 10 days after inoculation. Lesions active at time of death.
d Lesions were present at the same time on the opposite side of the comb. All lesions may therefore be due to accidental infection. If the lesions on the opposite side of the comb had resulted from inoculation, they would not have appeared until after the lesion at the point of inoculation.
e Lesions did not develop until the 24th day after vaccination. May not have been caused by the vaccine.
f These lesions not observed until the 28th day after vaccination. May have resulted from accidental infection rather than vaccination.

tissue. The decrease in amount after this time appeared to be the result of the beginning of dry-scab formation. Assuming that the tissue from these birds is of equal value for vaccine preparation, the most suitable time for securing tissue is shown to be when the lesions have reached their maximum development and before drying has begun. In this case, it was on the twelfth day after inoculation.

First Immunizing and Virulence Tests of Vaccines 7 to 16.—For the determination of the immunizing properties of the vaccines, groups of from two to four fowls were given a subcutaneous injection of vaccine and inoculated with chicken-pox virus 28 days later. Since the results of previous experiments had indicated that one dose of vaccine was as effective as two doses given seven days apart, one dose only was used in this experiment.

In all tests of the blood-and-organ-tissue vaccine, the dose was 1 cc. or 2 cc. of the vaccine as prepared. Each cubic centimeter contained 0.33 grams of tissue. The dose of skin-and-lesion-tissue vaccine was 1 cc. containing 0.1, 0.03, 0.01, 0.005, 0.002, or 0.001 grams of tissue. The variation in amount of tissue was accomplished by diluting the original preparation with glycerine-phenolized saline mixture so that 1 cc. of the dilution contained the amount of tissue it was desired to administer. The dilutions were always made just before use.

In the virulence tests one cockerel was inoculated with the same concentrations of vaccine used in the immunizing tests.

The results of the first tests are given in table 4.

As indicated in table 4, chicken-pox lesions that appeared on some of the birds that may have resulted from infection with virus other than that contained in the vaccine. Evidence that such virus was present is furnished by the fact that chicken-pox occurred among normal cockerels that were in another compartment of the same house. The irregularity of the occurrence of chicken-pox lesions on some of the birds with respect to the time of vaccination or inoculation provided an additional reason for believing that infection of some of the birds was accidental. Accurate interpretation of the results of these experiments is, therefore, rendered difficult, and for this reason they will not be discussed in detail.

However, the results of this experiment have again demonstrated that vaccine prepared from fresh lesion tissue and containing virulent chicken-pox virus when injected subcutaneously in fowls is capable of producing immunity to artificial infection with chicken-pox virus. The injection is liable to be followed by the occurrence at the point of injection of chicken-pox lesions which are slight and do not constitute a harmful infection. The significance of such lesions in the development of immunity is still undetermined since, in preceding experiments, immunity was produced without the occurrence of them. The smallest amount of lesion tissue that will immunize remains unknown since 0.001 gram was as effective in this respect as 0.1 gram.

The results with vaccines prepared from blood and internal organ tissue suggest that they contained virulent chicken-pox virus and possessed immunizing properties. However, on account of the occurrence on some of the experimental birds of chicken-pox lesions that apparently resulted from accidental infection, definite conclusions could not be drawn.

Second Immunizing and Virulence Tests of Vaccines 7 to 16.—To avoid interference from accidental infection of the experimental fowls such as was encountered in the preceding experiment, these tests were carried out under more carefully controlled conditions.

The results of the tests are given in table 5.

The data, as given in table 5, show that the 82 to 98-day-old vaccines 7 to 11, which were prepared from the blood, liver, spleen, and kidney tissue of cockerels that had marked chicken-pox lesions and were killed on the tenth to the fourteenth day after inoculation, contained no virulent chicken-pox virus and possessed no immunizing properties. The vaccines of the same age prepared from lesion tissues of the same birds, as shown in table 5, did, however, contain living virus and immunizing properties, although the doses of these vaccines contained but from one-tenth to less than one-three-hundredth as much tissue as the doses of vaccines 7 to 11. These results tend to substantiate the observations made in the first tests of vaccines 7 to 11, that the immunity of vaccinated birds and the lesions on the inoculated birds might be due to accidental infection rather than to the vaccines. These results also suggest that the immunizing properties of preceding vaccines which contained both types of tissue were due entirely to their lesion-tissue content. For these reasons the inclusion of blood, liver, spleen, and kidney tissue in the experimental vaccines was discontinued.

TABLE 5

RESULTS OF THE SECOND IMMUNIZING AND VIRULENCE TESTS OF VACCINES 7 TO 16

	Vaccine			Immunizing tests							Virulence tests[a]			
No.	Type of tissue	Age (days)	Amount of tissue in dose (grams)	Number of fowl vaccinated	Number that developed vaccination-point lesions	Number that developed lesions on the head	Number that were tested for immunity 28 days after vaccination	Number that were immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Age of vaccine (days)	Number inoculated	Lesions produced	Incubation period (days)
7	Blood and organs	98	0.33	4	0	0	3[b]	Marked lesions on 3	4	Marked lesions	86	6	None[c]	....
8	Blood and organs	97	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	85	6	None[c]	....
9	Blood and organs	96	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	84	6	None[c]	....
10	Blood and organs	95	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	83	6	None[c]	....
11	Blood and organs	94	.33	4	0	0	4	Marked lesions on 4	4	Marked lesions	82	6	None[c]	....
12	Lesion tissue	98	.03	4	0	0	4	Marked lesions on 3 Moderate lesions on 1	4	Marked lesions	86	6	Marked	5
13	Lesion tissue	97	.01	4	4	*0	3[b]	3 immune	4	Marked lesions	85	6	Marked	5
14	Lesion tissue	96	.005	4	4	0	4	4 immune	4	Marked lesions	84	6	Marked	5
15	Lesion tissue	95	.002	4	4	0	4	4 immune	4	Marked lesions	83	6	Marked	5
16	Lesion tissue	94	0.001	4	3[d]	0	4	3 immune Mild lesions on 1[d]	4	Marked lesions	82	6	Marked	5

[a] The amount of tissue in 1 cc was the same as in the corresponding immunizing test.
[b] One died from causes not related to vaccination.
[c] Subsequent inoculation of these birds with chicken-pox virus showed all of them to be susceptible to the infection.
[d] The bird that did not develop vaccination lesions is the one that was not immune.

All of the thirty cockerels inoculated with the 82 to 86-day-old vaccines 12 to 16 developed marked chicken-pox lesions. In all instances, the lesions became visible after an incubation period of five days. No differences in the amount of virulence of the virus was detected.

In the immunizing tests with these vaccines, however, the results were not so uniform. None of the four birds that received vaccine No. 12 developed vaccination lesions or were completely immune to infection 28 days after vaccination, but all except one of the sixteen birds that received vaccines 13, 14, 15, or 16 developed lesions at the point of injection of the vaccine and were immunized. Since the virulence tests of these vaccines, which were started 12 days earlier, indicated that their immunizing properties should be approximately the same, the failure of vaccine No. 12 to immunize may be due to a decrease in the virulence of its virus content during the 12-day interval. The results of the third virulence test of these vaccines, which is described later, indicate that this explanation is probably correct.

As previously stated, fifteen of the sixteen birds that received a dose of vaccine No. 13, 14, 15, or 16, containing 0.01, 0.005, 0.002, or 0.001 gram of tissue developed vaccination-point lesions and became immunized to chicken-pox infection. The bird that did not become absolutely immune to chicken-pox was that one of the four that received a dose of vaccine No. 16, containing 0.001 gram of tissue, and that did not develop vaccination-point lesions. This is the first instance in the series of experiments to indicate that the occurrence of lesions at the point of vaccination may be essential for the development of complete immunity. The failure of one of the four birds vaccinated with vaccine No. 16 to become completely immunized to infection with chicken-pox virus cannot be said to definitely indicate that this vaccine was less effective than vaccines 13, 14, or 15; the difference in the results may have been due to the smaller amount (0.001 gram) of tissue in a dose of vaccine No. 16 than the amounts (0.01, 0.005, or 0.002 gram) of tissue in the other vaccines.

Third, Fourth, and Fifth Virulence and Immunizing Tests of Vaccines 12 to 16.—In these tests, dilutions of vaccines 12, 13, 14, 15, and 16 containing 0.05, 0.01, 0.005, 0.002, and 0.001 gram of tissue per cubic centimeter, respectively, were used. The virulence tests were made first. An immunity test of a vaccine was not made when the virulence test indicated that the virus in it was no longer virulent. The manner of making the tests was the same as in preceding experiments. Table 6 gives the age of the vaccines when the tests were made, the number of birds used, and the results obtained.

TABLE 6

RESULTS OF THIRD, FOURTH, AND FIFTH VIRULENCE AND IMMUNIZING TESTS OF VACCINES 12, 13, 14, 15, AND 16

Vaccine No.	Test No.	Vaccine Age (days)	Amount of tissue in 1 cc (grams)	Immunising tests — Number of fowls vaccinated	Number that developed vaccination-point lesions	Number that developed lesions on the head	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Virulence tests — Age of vaccine (days)	Number inoculated	Lesions produced	Incubation period (days)
12	3a	....	....	....	....	....	....	....	....	....	128	3	None	....
13	3	140	0.01	4	4	0	4	4 immune	4	Marked	127	3	Marked lesions on all	6
	4	184	.01	4	0	0	3b	1 immune / Slight lesions on 2	2	Marked	194	2	Two tumors on each bird	7
	5	337	.1	3	0	0	3	Marked lesions on 3	2	Marked	320	2	None	....
14	3	139	.005	4	4	0	4	4 immune	4	Marked	126	3	Marked lesions on all	6
	4	183	.005	4	4	0	2c	2 immune	2	Marked	183	2	Both marked lesions	7
	5	336	.1	8	0	0	8	Marked lesions on 8	2	Marked	319	2	2 tumors on 1 bird / 1 tumor on 1 bird	7

a Since the virulence test was negative, no immunising tests with this vaccine were made.
b One died from causes not related to vaccination.
c Two died from causes not related to vaccination.
d Three died from causes not related to vaccination.
• One chicken-pox tumor developed on the wattle on the 28th day after vaccination.

TABLE 6—(Continued)

Vaccine											Immunising tests								Virulence tests			
Vaccine No.	Test No.	Age (days)	Amount of tissue in 1 cc (grams)	Number of fowls vaccinated	Number that developed vaccination lesions	Number that developed lesions on the head	Number tested for immunity 28 days after vaccination	Number immune or that developed chicken-pox lesions	Number of controls inoculated	Lesions produced	Age of vaccine (days)	Number inoculated	Lesions produced	Incubation period (days)
15	3	138	.002	4	3	0	3[b]	3 immune	4	Marked	125	3	Marked lesions on all	6
15	4	182	.002	4	0	0	4	3 immune; Slight lesions on 1	2	Marked	182	1	Marked lesions	7
15	5	335	.1	8	0	0	5[d]	2 immune; marked lesions on 2; Slight lesions on 1	2	Marked	318	2	Moderate lesions on both	7
16	3	137	.001	4	3	0	4	4 immune	4	Marked	124	3	Marked lesions on all	6
16	4	181	.001	4	2	1*	4	4 immune	2	Marked	181	2	1 tumor on each bird	7
16	5	334	0.1	8	0	0	6[c]	4 immune; Marked lesions on 1; Slight lesions on 1	2	Marked	317	2	6 tumors on 1 bird; No lesions on 1 bird	7

[a] Since the virulence test was negative, no immunising tests with this vaccine were made.
[b] One died from causes not related to vaccination.
[c] Two died from causes not related to vaccination.
[d] Three died from causes not related to vaccination.
* One chicken-pox tumor developed on the wattle on the 28th day after vaccination.

The third virulence test showed that the 128-day-old vaccine No. 12 no longer contained virulent chicken-pox virus. Therefore, no further tests of this vaccine were made. ·

The other four vaccines, Nos. 13, 14, 15, and 16, produced marked lesions on all of the birds used for the virulence tests and immunity of all of fifteen birds that were vaccinated and tested for immunity 28 days later. From these results it would appear, therefore, that these vaccines had not decreased in virulence or immunizing properties during 137 to 140 days of aging. Lesions developed at the point of vaccination on all but two of the sixteen birds vaccinated. Both of these birds, however, were immunized by the vaccine. This further demonstrates that the immunity to chicken-pox may be produced by vaccination without the production of visible lesions.

In the fourth test made when the vaccines were from 181 to 184 days old, vaccine No. 13, which contained the largest amount (0.01 gram) of tissue per cubic centimeter, caused but very slight lesions on the two cockerels used in the virulence test and absolute immunity of but one of three birds with which the immunizing tests was completed. The other two birds used in the latter test were resistant to infection but not absolutely immune. Vaccination lesions occurred on none of the birds. The lesions produced in the virulence test of vaccine No. 16, which contained the smallest amount (0.001 gram) of tissue per cubic centimeter, were no less pronounced than in the corresponding test of vaccine No. 13. All of four cockerels vaccinated with vaccine No. 16, however, became immunized. Vaccines 14 and 15, which contained 0.005 and 0.002 gram of tissue per cubic centimeter, respectively, produced marked lesions on the birds used in the virulence tests and immunity of five or six birds vaccinated. The lesions that developed on the bird that was not immune were of little consequence. Two other birds that were vaccinated were lost to the experiment by death.

These results are not entirely in harmony. Since vaccines 13 and 16 were found to contain but little virulent virus, while vaccines 14 and 15 possessed an abundance, it would be expected that vaccines 13 and 16 would be less effective immunizing agents than vaccines 14 and 15. This appeared to be true of vaccine No. 13 but vaccine No. 16 was more effective in this respect than vaccine No. 15 and just as effective as vaccine No. 14. Vaccines 14 and 15 appeared to be of equal virulence but the latter failed to immunize as consistently as the former. It would seem, therefore, that while the presence of virulent virus in vaccine is necessary for the production of immunity, the

extent of the lesions produced in virulence tests is not an absolute index of the immunizing property of vaccine.

The fifth virulence tests of vaccines 13, 14, 15, and 16, which were made when the vaccines were from 317 to 320 days old, indicated that no living virus remained in vaccine No. 13 and that the amount of living virus in the other vaccines was small. In the immunity tests, therefore, which were made 17 days later, instead of doses of vaccine varying in tissue content from 0.001 to 0.1 gram, the amount of tissue in a dose was 0.1 gram for all vaccines. None of eleven birds vaccinated with Nos. 13 and 14 were immunized. Six of eleven birds with which immunizing tests of vaccines 15 and 16 were completed became immune. The remaining five birds vaccinated with these two vaccines developed slight to marked lesions after inoculation with chicken-pox virus 28 days later. These results show that these vaccines had lost most of their virulence and immunizing properties and, therefore, no further work with them was done.

This series of experiments with ten vaccines, five of which (Nos. 7, 8, 9, 10, and 11) were prepared from the blood, liver, spleen, and kidneys, and five of which (Nos. 12, 13, 14, 15, and 16) were prepared from the lesion tissue has failed to show definitely that the vaccines prepared from the blood-and-organ tissue contained virulent virus or possessed immunizing properties. The lesion-tissue vaccines, however, were shown to contain an abundance of virulent virus and, when injected subcutaneously, were shown to be capable of establishing an immunity to artificial infection with chicken-pox virus within 28 days. The use of doses of vaccine containing amounts of tissue varying from 0.1 to 0.001 gram did not indicate that a dose containing the largest amount was more effective than one containing the smallest amount.

Forty-two of the sixty-five fowls that became immunized developed chicken-pox lesions at the point of injection after vaccination. The fowls that did not develop such lesions were principally from those vaccinated after the vaccine had aged 180 days or more. That the vaccination point of all immunized fowls did not become infected is further evidence that such infection is not essential to immunization. In this and all preceding experiments, however, it has been observed that all fowls that developed vaccination lesions did become immune. Such lesions have proved harmless, in so far as their effect on the general health of the fowls or tendency to initiate more widespread infection is concerned. Therefore, they may be regarded as a vaccination 'take' indicating that immunization will be accomplished, and as a favorable reaction to vaccination with fresh-lesion-tissue vaccine.

As previously stated, the purpose of securing material for the preparation of these vaccines from cockerels that were killed on the tenth, eleventh, twelfth, thirteenth, and fourteenth days, respectively, after inoculation with chicken-pox virus, was to obtain data regarding the most suitable time after inoculation to secure tissue for vaccine preparation. From the standpoint of yield of tissue per bird the 12-day lesion rank first, the 13, 14, 11 and 10-day lesions following in the order named.

In the tests of the fresh vaccines, no differences between them were detected. The tests made when the vaccines were from 94 to 98 days old indicated that vaccine No. 12, prepared from 10-day lesions, had decreased considerably in immuzing properties. The other four vaccines were apparently unchanged. The third test, made when the vaccines were from 127 to 140 days old, showed that vaccine No. 12 was entirely impotent, while immunizing properties of the others were apparently undiminished. At the fourth test, made when the vaccines were 181 to 184 days old, it was found that vaccine No. 13, prepared from 11-day lesions, had lost most of its virulence and ability to immunize, but that the other three were still active in these respects. The fifth test was made when the vaccines were from 334 to 337 days old. Vaccine No. 13 had lost all of its virulence and immunizing properties. Vaccines 14, 15, and 16 still contained some virulent virus but were poor immunizing agents. The vaccines prepared from chicken-pox lesions removed on the twelfth, thirteenth, and fourteenth days after inoculation were shown to retain their virulence and immunizing property longer than those prepared from lesions of cockerels killed 10 or 11 days after inoculation. The cockerels killed on the twelfth, thirteenth, and fourteenth days after inoculation also, as shown previously, yielded more lesion tissue than those killed earlier. These results, therefore, suggest that the most suitable time for securing material for the preparation of fresh lesion tissue vaccine is on the day that the lesions have attained maximum development, or one or two days after. When highly virulent virus is used for inoculation, the time between inoculation and the proper time for removal of the lesions would probably not vary more than one or two days, from the 12 to 14-day period of these experiments.

EXPERIMENTS WITH VACCINES PREPARED FROM LESION TISSUE

These experiments consist of immunizing and virulence tests of a series of five vaccines (Nos. 20, 21, 23, 24, and 25) prepared from fresh lesion tissue in the same manner as vaccines 12 to 16 in the preceding experiment. A brief description of the vaccines follows.

Vaccines 20 and 21 were prepared on March 29 and April 28, 1926, respectively, from lesions removed from cockerels on the twelfth day after vaccination. Each cubic centimeter of these vaccines contained 0.1 gram of tissue. Most of the immunizing and virulence tests of these vaccines were made with dilutions containing 0.005, 0.002, and 0.001 gram of tissue per cubic centimeter. The dilutions were always made immediately before use. Virulence tests were made of vaccine No. 20 when it was 15, 42, 51, 61, 71, and 219 days old. Immunizing tests were made when it was 29, 51, 66, and 236 days old. The virulence tests of vaccine No. 21 were made when it was 13, 55, and 219 days old, and the immunizing tests when it was 55 and 236 days old.

Vaccines 23, 24, and 25 were prepared from 12-day lesions on June 14, June 24, and August 31, 1926, respectively. Each cubic centimeter of these vaccines contained 0.25 gram of tissue. The virulence and immunizing tests were mainly of dilutions containing 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter. Vaccine No. 23 was tested for virulence when it was fresh and 36 days and 142 days old, and for immunizing properties when it was fresh and 161 days old. Virulence tests of vaccine No. 24 were made with 86 and 182-day-old vaccine. Virulence tests of vaccine No. 25 were made on the seventh, thirtieth, and sixty-fourth days, and immunity tests on the seventh, thirtieth, and seventy-first days after its preparation.

The number of birds used and other details regarding the tests are given in table 7.

As shown in table 7, there was so little difference between the results of the immunizing and virulence tests of the five vaccines that they may be discussed as a whole instead of separately.

The virulence tests may be grouped as those with vaccine from 1 to 75 days old and as those with vaccines from 132 to 219 days old. No tests were made with vaccines between the ages of 75 and 132 days.

TABLE 7

RESULTS OF THE IMMUNIZING AND VIRULENCE TESTS OF VACCINES 20, 21, 23, 24, AND 25

Immunizing tests

Vaccine No.	Test No.	Vaccine Age (days)	Amount of tissue in 1 cc (grams)	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions
20	1	29	0.005	4	4	0	4	28	4 immune
			.002	4	4	0	4	28	4 immune
			.001	4	3	0	4	28	4 immune
20	2	51	.005	4	3	0	4	28	4 immune
			.002	4	4	0	4	28	4 immune
			.001	4	3	0	4	28	4 immune
20	3	66	.002	125	97	0	125	28	117 immune; 8[a] slight lesions
20	4	236	.1	4	0	0	4	62	4 marked lesions

Virulence tests

Test No.	Age of vaccine (days)	Tissue in 1 cc of vaccine (grams)	Number inoculated	Lesions produced	Incubation period (days)
1	15	0.01	3	Marked	5
		.005	3	Marked	5
		.002	3	Marked	5
		.001	3	Marked	5
2	43	.005	2	Marked	6-7
		.002	2	Marked	6-7
		.001	2	Marked	6
3	51	.005	2	Marked	7
		.002	2	Marked[b]	8
		.001	2	Marked	8
4	61	.005	2	Marked	7
		.002	2	Marked	7
		.001	2	Marked	7
5	71	.005	2	Marked	7
		.002	2	Marked	7
		.001	2	Marked	7
6	219	.1	2	None	...

[a] Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
[b] Lesions not as marked as those on birds inoculated with .002 gram dilution.
[c] Eight died from infectious bronchitis.
[d] One died from infectious bronchitis.
[e] Three died from undetermined cause.
[f] One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
[g] Lesions healed in from 12 to 14 days after inoculation.

TABLE 7—(Continued)

		Immunizing tests								Virulence tests					
Vaccine No.	Test No.	Vaccine Age (days)	Vaccine Amount of tissue in 1 cc (grams)	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vaccine (days)	Tissue in 1 cc of vaccine (grams)	Number inoculated	Lesions produced	Incubation period (days)
21	1	55	.005	4	4	0	4	28	4 immune	1	13	.005	2	Marked	6
			.002	10	8	0	2[a]	28	2 immune			.002	2	Marked	6
			.001	4	4	0	3[d]	28	3 immune			.001	2	Marked	6
21	2	206	.1	4	0	0	3[d]	62	3 immune	2	55	.005	2	Marked	7
			.005	4	0	0	4	62	4 immune			.002	2	Marked	7
			.002	4	0	0	3	62	3 immune			.001	2	Marked	7
			.001	4	0	0	3	62	3 immune	3	189	.1	2	No lesions on one / 6 tumors on one	12
												.005	2	None	....
												.002	2	One tumor on one / No lesions on one	12
												.001	2	One tumor on one	12
23	1	Fresh	.005	10	7[e]	0	7[e]	28	7 immune	1	Fresh	.005	2	Marked	6
			.002	10	9	0	10	28	10 immune			.002	2	Marked	6
			.001	10	9	0	7[e]	28	5 immune / 2 slight lesions[f]			.001	2	Marked	6

a Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
b Lesions not as marked as those on birds inoculated with .002 gram dilution.
c Eight died from infectious bronchitis.
d One died from infectious bronchitis.
e Three died from undetermined cause.
f One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
g Lesions healed in from 12 to 14 days after inoculation.

TABLE 7—(Continued)

Vac-cine No.	Immunising tests									Virulence tests					
	Test No.	Vaccine Age (days)	Amount of tissue in 1 cc (grams)	Number of fowls vaccin-ated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Num-ber tested for im-munity	Days after vaccina-tion when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vac-cine (days)	Tissue in 1 cc of vac-cine (grams)	Num-ber inocu-lated	Lesions produced	Incu-bation period (days)
23	2	161	.005	4	0	0	3[d]	62	2 immune	2	36	.002	2	Marked	7
			.002	4	0	0	4	62	1 slight lesion[e]	3	142	.25	2	Marked	7
			.001	4	0	0	4	62	4 slight lesions[e]			.005	2	Marked	7
												.002	2	Moderate lesions on 1	7
	1	86	.002	3	1	0	3	32	3 immune	1	75	.001	2	6 tumors on one	7
														No lesions on one	
														Moderate lesions on 1	
24	2	151	.005	4	0	0	4	62	2 immune	2	132	.25	2	Marked	6
			.002	4	0	0	4	62	2 slight lesions[f]			.005	2	Marked	6
			.001	4	0	0	4	62	4 immune			.002	2	Marked lesion on one	7
	1						3	62	3 immune			.001	2	One tumor on one	7
								62	1 slight lesion[g]					No lesions on one	
														Three tumors on one	

[a] Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.
[b] Lesions not as marked as those on birds inoculated with .002 gram dilution.
[c] Eight died from infectious bronchitis.
[d] One died from infectious bronchitis.
[e] Three died from undetermined cause.
[f] One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.
[g] Lesions healed in from 12 to 14 days after inoculation.

TABLE 7—(Concluded)

Vaccine No.	Test No.	Vaccine — Age days	Vaccine — Amount of tissue in 1 cc grams	Number of fowls vaccinated	Number that developed lesions at vaccination-point	Number that developed lesions on the head	Number tested for immunity	Days after vaccination when tested	Number immune or that developed chicken-pox lesions	Test No.	Age of vaccine days	Tissue in 1 cc of vaccine grams	Number inoculated	Lesions produced	Incubation period days
25	1	7	.005	4	4	0	4	30	4 immune	1	7	.005	2	Marked	6
			.002	4	4	0	4	30	4 immune			.002	2	Marked	6
			.001	4	4	0	4	30	4 immune			.001	2	Marked	6
25	2	30	.005	4	4	0	4	28	4 immune	2	30	.005	2	Marked	6
			.002	4	4	0	4	28	4 immune			.002	2	Marked	6
			.001	4	4	0	4	28	4 immune			.001	2	Marked	6
25	3	71	.005	4	4	0	4	32	4 immune	3	64	.005	2	Marked	6
			.002	4	4	0	4	32	4 immune			.002	2	Marked	6
			0.001	4	4	0	4	32	4 immune			0.001	2	Marked	6

a Four of these had vaccination-point lesions—four had not. All lesions healed in 15 days after inoculation.

b Lesions not as marked as those on birds inoculated with .002 gram dilution.

c Eight died from infectious bronchitis.

d One died from infectious bronchitis.

e Three died from undetermined cause.

f One of these had vaccination-point lesions—one had not. All lesions healed within 18 days after inoculation.

g Lesions healed in from 12 to 14 days after inoculation.

Eighty birds were used in virulence tests of 1 to 75-day-old vaccine. Three were inoculated with vaccine containing 0.01 gram of tissue per cubic centimeter, twenty-five with vaccine containing 0.005 gram of tissue per cubic centimeter; twenty-seven with vaccine containing 0.002 gram of tissue per cubic centimeter, and twenty-five with vaccine containing 0.001 gram of tissue per cubic centimeter. In one instance, that of the two birds inoculated with 51-day-old vaccine, No. 20, the lesions produced by inoculation with the 0.001-gram dilution were less pronounced than those on the birds inoculated with the dilutions containing more tissue. Otherwise the lesions appeared to be of equal severity irrespective of the tissue content of the vaccines. The lesions appeared after an incubation period of from 5 to 8 days and were evenly distributed over the scarified area. By the twelfth or fourteenth day the lesions were very pronounced and covered an area one and one-half to two times as large as that scarified. The incubation period showed a tendency to increase slightly with the age of the vaccine. With vaccine No. 20, the increase was from 5 days for 15-day old vaccine to 8 days for 51-day-old vaccine and 7 days for 61-day-old vaccine. With vaccine No. 21, the incubation period was 6 days for fresh vaccine and 7 days for 55-day-old vaccine. With 7, 30, and 64-day-old vaccine No. 25, the incubation period was uniformly 6 days. Since the incubation period did not in all cases increase as the vaccine increased in age, it would seem just as probable that the slight differences in periods of incubation, after inoculation with the 1 to 75-day-old vaccine, were due to differences in the susceptibility of the birds as they were due to changes in the virus.

In contrast to the uniform results of virulence tests of different dilutions of the 1 to 75-day-old vaccine, the different dilutions of the 132 to 219-day-old vaccines did not produce lesions of the same degree of severity. Inoculation with dilutions of these vaccines containing 0.25 and 0.005 gram of tissue resulted in the development of pronounced lesions. The birds inoculated with dilutions containing 0.002 or 0.001 gram of tissue, however, developed either no lesions at all or only a few discrete tumors. These separate tumors were as persistent and became proportionately as pronounced as the more extensive lesions. The period of incubation was in all instances 6 or 7 days. It would seem, therefore, that the change that had occurred in the virus content as a result of aging was more probably a decrease in amount due to the death of part of the virus than to a decrease in virulence.

Inoculation with dilutions of 189-day-old vaccine containing 0.1, 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter produced

either no lesions or a few discrete tumors. The period of incubation was 12 days. These results indicate that the virus in this vaccine had decreased in both amount and virulence.

Inoculation with 219-day-old vaccine containing 0.1 gram of tissue per cubic centimeter produced no lesions. From this it was concluded that all of the virus in the vaccine had been destroyed.

The immunizing tests may be divided into those of vaccines from 1 day to 86 days old and those of vaccines after they were from 151 to 236 days old. No tests were made with vaccines between 86 and 151 days old.

Immunizing tests of 1 to 86-day-old vaccines were completed with 221 birds. The tissue content of a dose of vaccine was 0.005, 0.002, or 0.001 gram. The results are summarized in table 8.

TABLE 8

SUMMARY OF RESULTS OF IMMUNIZING TESTS WITH 1 TO 86-DAY-OLD VACCINES
20, 21, 23, 24, AND 25

Grams of tissue in dose of vaccine	Number of fowls vaccinated	Number that developed vaccination-point lesions	Number that were immune
0.005	31	30	31
0.002	160	129	152
0.001	30	27	28
Totals..........	221	186	211

Table 8 shows that thirty-five of the 221 fowls that were vaccinated failed to develop a vaccination lesion or 'take.' The distribution of these birds is as follows:

   1 was vaccinated with the 0.001 gram dilution of 29-day-old vaccine No. 20
   1 was vaccinated with the 0.005 gram dilution of 51-day-old vaccine No. 20
   1 was vaccinated with the 0.001 gram dilution of 51-day-old vaccine No. 20
28 were vaccinated with the 0.002 gram dilution of 66-day-old vaccine No. 20
   1 was vaccinated with the 0.002 gram dilution of fresh vaccine No. 23
   1 was vaccinated with the 0.001 gram dilution of fresh vaccine No. 23
   2 were vaccinated with the 0.002 gram dilution of 86-day old vaccine No. 24

This shows that the absence of vaccination lesions was not confined to any vaccine or to any age or dilution of vaccine. That there were more failures among the birds vaccinated with the 0.002-gram dilution of vaccine No. 20 may be explained by the fact that this dilution of vaccine No. 20 was given to more than three-fourths of the birds vaccinated.

The lesions of the ten birds which, as shown in table 8, were not entirely immunized consisted of the formation of a small amount of dry yellow scab over the scarified area. These lesions never had the appearance of an active chicken-pox lesion and were entirely healed in from 12 to 18 days after inoculation. The birds were eight of the one hundred and twenty-five that were vaccinated with the 0.002-gram dilution of the 66 day-old vaccine No. 20 and two of the seven birds that were vaccinated with the 0.001-gram dilution of fresh vaccine No. 23. These results cannot be interpreted as indicating that vaccine No. 20 was less effective than the others, since this vaccine was used on more than three-fourths of the birds. If the other vaccines had been used on an equal number of birds it is probable that some failures to confer complete immunity would have been encountered.

The ten birds that were not absolutely immunized were equally distributed among those that developed vaccination-point lesions and those that did not. This again demonstrates that immunity can develop in the absence of a lesion at the point of vaccination or other visible vaccination reaction. It also demonstrates that the occurrence of a lesion at the point of vaccination is not a positive indication of the development of complete immunity. It should be pointed out, however, that the number of the birds that were not entirely immune was proportionately greater among those that did not develop vaccination lesions than among those that did develop such lesions.

None of the forty birds with which, as shown in table 7, the immunizing tests of the 151 to 236-day-old vaccine were completed, developed vaccination lesions. Inoculation with chicken-pox virus sixty-two days after vaccination showed that fourteen of the twenty-three birds that received 151 or 161-day-old vaccine and all of ten birds that received 206-day-old vaccine were immune. The character of the lesions that were produced on the nine birds that were not immune indicated that they had a high degree of resistance to the infection. These results show that the 151 to 206-day-old vaccines were less efficient immunizing agents than they had previously been and are in agreement with the results of the virulence tests, which showed that the virus content of the vaccines had become reduced during the period of storage. The four birds vaccinated with the 236-day-old vaccine acquired neither immunity nor increased resistance to infection with chicken-pox virus. These results are in accordance with the virulence tests, which showed that the vaccine contained no virulent chicken-pox virus.

SUMMARY AND DISCUSSION OF THE RESULTS OF THE VACCINATION EXPERIMENTS

In the preliminary experiments it was demonstrated that by subcutaneous injections with vaccine prepared from the whole comb of cockerels with extensive chicken-pox lesions, fowls can be immunized against artificial infection with chicken-pox virus 28 days later. Equally good results were obtained with vaccine prepared from the whole comb, blood, liver, spleen, and kidneys of infected cockerels. Both of the above types of vaccine were shown to contain a virulent chicken-pox virus, but further comparative experiments with a vaccine · prepared with comb tissue and one prepared with the blood, liver, spleen, and kidney tissue of infected cockerels failed to demonstrate definitely that the latter type of vaccine possessed immunizing properties. The use of blood, liver, spleen, and kidney tissue in the experimental vaccines was therefore discontinued. The vaccine containing comb tissue retained its potency for as long as 86 days. One dose was as effective as two doses given 7 days apart. Small chicken-pox lesions developed on the skin of many fowls at the point where the vaccine was injected. These lesions did not spread to other parts nor otherwise prove harmful to the birds.

The fibrous portion of the comb tissue was with difficulty reduced to a fine state, much of it was removed by straining, and that portion of it remaining in the vaccine was thought to be inert. Therefore the plan was adopted of using only the lesion and sub-lesion tissue instead of the entire comb. Since this type of vaccine was considered more satisfactory than the preceding, the results obtained with it will be discussed in more detail. The lesion-tissue vaccines as prepared contained either 0.1 or 0.25 gram of tissue per cubic centimeter. The volume of a dose of vaccine was uniformly 1 cc. to each bird. Variation in the size of dose was accomplished by making dilutions of the original preparation containing different amounts of tissue per cubic centimeter. The dilutions that were used most extensively contained 0.005, 0.002, and 0.001 gram of tissue in a cubic centimeter. The age of the vaccines at the time of use varied from 1 day to 337 days. Because of the similarity of the results obtained with vaccines between certain age limits, it is possible to summarize the results as those obtained with vaccines from 1 day to 140 days old and those obtained with vaccines from 151 to 337 days old. Such a summary is given in table 9.

TABLE 9

SUMMARY OF RESULTS OF VACCINATION WITH LESION-TISSUE VACCINE

Age of vaccine *days*	Number of fowls vaccinated	Number immunised	Per cent immunised	Fowls that developed vaccination lesions				Fowls that did not develop vaccination lesions			
				Total number	Per cent of number vaccinated	Number immune	Per cent immune	Total number	Per cent of number vaccinated	Number immune	Per cent immune
1 to 140	275	263	95.6	234	85.0	229	97.8	41	14.9	34	82.9
151 to 337	74	43	58 1	4	5.4	4	100.0	70	94.5	39	55.7

As shown in table 9, the 1 to 140-day-old vaccines were highly efficient immunizing agents and caused the development of lesions at the point of vaccination on 85 per cent of the fowls. Thirty-four of the immune fowls were among those that did not develop vaccination lesions. This indicates that a vaccination lesion was not essential to the development of complete immunity. It is seen, however, that the percentage of fowls that were immunized is higher among those that developed vaccination lesions than among those that did not. This shows that a vaccination lesion may be regarded as an indication of the development of immunity and, therefore, as a favorable reaction after vaccination.

The inoculation of cockerels with the same dilutions of the 1 to 140-day-old vaccines as were used for vaccination produced marked chicken-pox lesions on the scarified comb surfaces. As previously shown, this vaccine, when injected subcutaneously, proved innocuous except for the production of slight lesions at the point of injection. This shows that the subcutaneous injection of birds with vaccine containing highly virulent virus is unlikely to produce harmful lesions. This is a factor of great importance in the production of chicken-pox vaccine such as is used in these experiments, since the immunizing property of such vaccine is dependent upon its virus content. The fact that vaccinated birds developed vaccination-point lesions indicate that they can transmit infection to susceptible fowls with which they might be associated. In the use of such vaccine on a poultry farm, therefore, it would be necessary that all susceptible fowls be vaccinated.

The results obtained with the 151 to 337-day-old vaccine, as shown in table 9, require little comment. Of the seventy-four fowls vaccinated but forty-three were immunized and only four developed vaccination-point lesions. These results indicate that the 151 to 337-day-old vaccines were less satisfactory immunizing agents than when they

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were from 1 to 140 days old. The virulence tests of these vaccines demonstrated that the amount of virus in all vaccines had been reduced during the aging periods and in some cases had been entirely destroyed.

THE LONGEVITY OF VIRULENCE OF VIRUS IN LESION-TISSUE VACCINE AS INFLUENCED BY THE PROPORTION OF TISSUE AND DILUENT

A vaccine was prepared from such amounts of lesion tissue and glycerine-phenolized-saline mixture as to make 1 cc. of the final product contain 0.25 gram of tissue. Seven dilutions of this vaccine with the glycerine-phenolized-saline mixture were immediately made. The seven dilutions contained 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, and 0.001 gram of tissue, respectively, in one cubic centimeter of vaccine.

TABLE 10

THE RESULTS OF TESTS TO SHOW THE EFFECT OF AGE UPON THE VIRULENCE OF THE VIRUS IN DILUTIONS OF VACCINE CONTAINING DIFFERENT AMOUNTS OF TISSUE PER CUBIC CENTIMETER

Test number	Age of vaccine *days*	Age of dilution *days*	Grams of tissue in one cc	Number inocu-lated	Lesions produced
2	31	31	0.25	2	Marked
		31	.001	2	None
		40	.25	2	Marked
		40	.10	2	Marked
		40	.05	2	Marked
3	40	40	.02	2	Marked
		40	.01	2	None
		40	.005	2	None
		40	.002	2	None
		40	.001	2	None
		Fresh	.01	2	Marked
4	50	Fresh	.005	2	Marked
		Fresh	.002	2	Marked
		Fresh	.001	2	Marked
		177	.25	2	Marked
		177	.1	2	2-4 tumors on each bird
		177	.05	2	None
		177	.02	2	None
		Fresh	.1	2	Marked
5	177	Fresh	.05	2	Moderate
		Fresh	.02	2	3 tumors on each bird
		Fresh	.01	2	1 tumor on each bird
		Fresh	.005	2	1 tumor on each bird
		Fresh	.002	2	2 tumors on each bird
		Fresh	0.001	2	None

These preparations were stored in an icebox and tested at intervals for the presence in them of virulent virus. The tests consisted of applying vaccine to a scarified surface of the combs of cockerels. Chicken-pox lesions were produced when the vaccine contained virulent virus. The first tests were made with fresh preparations and showed that each dilution contained an abundance of virulent virus. Other tests were made when the vaccine and dilutions were 31, 40, 50, and 177 days old. The results are given in table 10.

As shown in table 10, the results of the second virulence tests, which included only dilutions containing the highest (0.25 gram) and lowest (0.001 gram) concentrations of tissue, indicated that the 0.25-gram concentration contained virus of unimpaired virulence while that of the virus in the 0.001-gram dilutions was entirely lost during the 31 days of storage. These results led to the testing of all of the dilutions of vaccine at the age of 40 days. The results showed that virulent virus was still abundant in the dilutions containing 0.25, 0.1, 0.05, 0.02 gram of tissue per cubic centimeter, but failed to demonstrate the presence of virulent virus in the dilution containing 0.01, 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter. Fresh dilutions containing these latter amounts of tissue per cubic centimeter were then made from the 0.25-gram dilutions and tested for virulence. Marked chicken-pox lesions were produced in all instances. This demonstrated that the negative results of the virulence test of the 40-day-old dilutions were due to destruction of the virus during the period of storage. Therefore no further tests of the dilutions of vaccine containing 0.01, 0.005, 0.002, or 0.001 gram of tissue per cubic centimeter were made. The results of virulence tests that were made when the vaccine was 177 days old indicated that in the 0.02 and 0.05-gram dilution there remained no virulent virus, and in the 0.1-gram dilution but a small amount. The undiluted (0.25-gram) vaccine was found still to contain virulent virus. The results of virulence tests of fresh dilutions of this vaccine, however, indicated that the amount of virus in the undiluted vaccine was much less than it had been previously.

The results of this series of tests indicate that the longevity of the virulence of chicken-pox virus in fresh lesion-tissue vaccine decreases as the proportion of tissue to diluent decreases. It is therefore desirable, in the preparation of vaccine that is to be stored, to use only as much diluent as is necessary to facilitate the grinding process and to destroy contaminating bacteria. Dilutions that are used for vaccination should be made immediately before the vaccine is administered.

RESULTS OF THE USE OF LESION-TISSUE VACCINE IN
INFECTED FLOCKS

The preceding experiments have demonstrtaed that there is apparently little danger of inducing harmful chicken-pox infection by the subcutaneous injection of vaccine containing virulent chicken-pox virus.   All of the birds used in these experiments were healthy cockerels and were kept under good environmental conditions apart from other fowls.   It was not demonstrated, therefore, that such vaccine could with safety be administered to flocks in which chicken-pox existed or in which other diseases were present, or during periods of cold, wet, or otherwise unfavorable weather.   It was thought that the use of vaccine containing virulent virus under such conditions might increase the severity of the disease in infected birds or in birds that were in the incubation stage of disease at the time of vaccination, or result in extensive infection with chicken-pox among those birds that were free from infection at the time of vaccination.   To secure information on these points vaccine was administered to 3125 fowls in four flocks in which some of the fowls were infected with chicken-pox.   In flock No. 1, the infection was produced by adding a few diseased birds to a flock of cockerels.   These were kept in the houses that were used for experimental purposes.   The other three flocks were farm flocks in which chicken-pox from natural infection was occurring.   The results follow:

Flock No. 1.—One hundred and ninety-five healthy cockerels were confined with cockerels infected with chicken-pox until fifty-one, or 26.1 per cent, of the healthy had developed chicken-pox lesions. Seventy-six of the healthy birds were then vaccinated with a dose of vaccine containing 0.002 gram of tissue.   Tests showed that the vaccine contained an abundance of virulent chicken-pox virus.   Sixty-eight of the healthy birds were not vaccinated.   During the two weeks after vaccination, lesions appeared about the head of twenty-five, or 32.8 per cent, of those that were vaccinated, and twenty-nine, or 52.6 per cent, of those that were not vaccinated.   No additional fowls in either group became infected.   Lesions developed at the point of vaccination on thirty-nine, or 51.3 per cent, of the seventy-six birds vaccinated.   Only nine of these were among the twenty-five vaccinated fowls that developed lesions about the head.   This indicates that there was no relation between the occurrence of lesions at the point of vaccination and the occurrence of them about the heads of the fowls.

Lesions on the head did not become severe enough to cause death of any of the birds. All that became infected had recovered within 36 days after vaccination. It is evident, therefore, that neither the rate of spread of the infection among the birds nor the severity of the lesions on those that became infected was increased by vaccination.

Vaccine was administered to twenty-six of the fifty-one birds that had become infected before any of the healthy birds were vaccinated. The lesions on those vaccinated did not become any more severe and were no more persistent than the lesions on those that were not vaccinated.

Flock No. 2.—The second flock consisted of 858 hens of the White Leghorn, Rhode Island Red, and Barred Plymouth Rock breeds. They were vaccinated with vaccine containing 0.002 gram of tissue per cubic centimeter. It was demonstrated by tests that the vaccine .contained an abundance of virulent virus. Seventeen fowls had become infected prior to vaccination. There were two additional cases of chicken-pox in the flock after vaccination, the last case developing on the twenty-third day. None of the fowls died from this cause. During this period infectious bronchitis became prevalent, causing a sickness of approximately one-third and the death of about 7 per cent of the fowls. The weather at the time of and after vaccination was generally cold and damp.

Flock No. 3.—The third flock consisted of approximately 1,700 White Leghorn pullets. The dose of vaccine given contained 0.002 gram of tissue. The presence of virulent virus in the vaccine was determined by virulence tests. The vaccine was administered and all observations of results were made by the owner of the flock. Detailed data regarding the condition of this flock were not obtained. It is known, however, that many of the birds were infected with chicken-pox and that losses were occurring from infectious bronchitis and ruptured yolk. The weather was cold and wet. The owner stated that the loss from chicken-pox was slight and that all evidence of that disease disappeared in about three weeks after vaccination.

Flock No. 4.—The fourth flock consisted of 372 White Leghorn hens, forty-nine of which had chicken-pox lesions. Two hundred of the healthy birds were vaccinated and one hundred and fifteen were left unvaccinated for controls. After vaccination, thirty-three, or 16.5 per cent, of the vaccinated group and thirty-six, or 31.3 per cent, of the control group developed the disease. The last case of chicken-pox in the control group occurred on the twenty-eighth day after vaccination and all except three of the thirty-three cases in this group

occurred before the seventeenth day after vaccination. In the control group, thirteen of the thirty-six cases of chicken-pox occurred after the twenty-eighth day and seventeen of the cases after the seventeenth day after the date of vaccination.

From the results obtained with these four flocks it is seen that the administration of vaccine containing virulent chicken-pox virus to flocks in which chicken-pox or other diseases were present and when cold and wet weather prevailed had no harmful effects.

FIELD TRIALS WITH VACCINE PREPARED FROM FRESH LESION TISSUE

These trials were carried out on poultry farms on which chicken-pox had been prevalent in the past but on which little or no active infection existed at the time the trials were instituted. They consisted in the vaccination of the pullets and cockerels when they were from four and one-half to six months old and before any of them had become infected with chicken-pox. After vaccination individual examinations of the fowls were made to detect those on which chicken-pox lesions developed either at the point of injection of the vaccine or about the head. In these trials it was possible to leave only a few birds unvaccinated as controls, since they were carried out on poultry farms and the owners were unwilling to risk the loss incident to infection that might occur among the fowls that were not vaccinated.

Virulence and immunizing tests of each lot of vaccine used in the field trials were made at the laboratory. By these means it was determined that all vaccines used in the field trials possessed an abundance of virulent virus and would immunize fowls against artificial infection with chicken-pox virus 28 days after vaccination. Five different vaccines, Nos. 20, 21, 23, 24, and 25, were used. They were prepared in an identical manner but varied with respect to their tissue content, Nos. 20 and 21 containing 0.1 gram of tissue per cubic centimeter, and Nos. 23, 24, and 25 containing 0.25 gram of tissue per cubic centimeter. The vaccine was diluted sufficiently at the time of use so that the dose was uniformly 1 cc. containing 0.002 gram of tissue. The age of the vaccines at the time of use varied from 31 to 85 days.

The primary purpose of the field trials was to determine if young healthy fowls on poultry farms could be vaccinated with lesion-tissue vaccine containing virulent chicken-pox virus without danger of producing serious chicken-pox infection among them. It was hoped that information might also be obtained regarding the extent to which the

fowls were protected against natural infection during the winter period after vaccination. It was realized, however, that, since the trials were uncontrolled, failure of chicken-pox to occur could not with certainty be attributed to protection afforded by vaccination, even though chicken-pox virus was undoubtedly present on the premises on which the fowls were kept. An additional feature of the field trials was to obtain information regarding the length of immunity produced by vaccination. For this purpose fowls were secured at varying intervals after vaccination, their combs scarified, and chicken-pox virus applied to the scarified surface.

The description and results of the field trials follow:

Field Trial No. 1.—On the farm at which field trial No. 1 was conducted chicken-pox had been continuously present for more than a year. At the time this trial was started (April 30, 1926) the disease was confined to a few hens in one small laying house, and to a few of a lot of 600 two to three-month-old pullets and cockerels. All of the infected and contact birds were well isolated from the remainder of the flock and the outlook was that the spread of the disease had been checked for the time. All of the young stock was reared on clean range at a considerable distance from the rest of the flock. However, when they reached the age of from four to five months they were transferred to laying houses and yards that had recently been occupied by infected flocks. The probability that some of the birds would become infected from contact with chicken-pox virus in the laying houses was therefore great.

The plan of vaccinating the birds as they were moved from the range to the laying houses was adopted. Since the birds were hatched at various times from January to April, they were moved to the laying houses in twelve lots between April 30 and September 30. Slightly more than half of the first lot of birds were left unvaccinated. All of the birds in the other lots were vaccinated. At least two examinations of each bird were made during the first four weeks after vaccination. The total number of birds vaccinated was 9,361. By means of colored leg bands the birds were marked so that those that developed vaccination lesions could later be distinguished from those that did not. The colored bands also served to identify the birds according to the lot to which they belonged and the date upon which they were vaccinated.

Field Trial No. 2.—The second field trial was carried out on a poultry farm on which an outbreak of chicken-pox had occurred during each winter for the last five years. There were no infected birds on the premises when this trial was started (September 17,

1926).   All of the pullets and cockerels that were hatched during 1926 were vaccinated.   They consisted of 799 White Leghorn and Barred Plymouth Rock cockerels from three to seven months old, 188 White Leghorn and Barred Plymouth Rock pullets from four to five months old, 340 White Leghorn pullets from six to seven months old, and 205 Barred Plymouth Rock pullets from six to seven months old. The weight of the birds varied from less than two pounds in the case of some of the young Leghorn pullets and cockerels to seven or eight pounds in the case of some of the Rock cockerels.   The dose of vaccine, however, was uniformly 1 cc. containing 0.002 gram of tissue.   The six to seven-month-old Leghorn and Rock pullets were producing approximately sixty eggs for each one hundred birds daily at the time of vaccination.

The results of the field trials are summarized in table 11.

TABLE 11

RESULTS OF FIELD TRIALS OF VACCINE PREPARED FROM FRESH LESION TISSUE
Birds were White Leghorns from four to five months old unless otherwise noted.
Dose—1 cc. containing 0.002 gram tissue.

Lot No.[a]	Vaccine		Number vaccinated	Birds that developed lesions at point of vaccination		Birds that developed lesions on the head	
	No.	Age					
		days		Number	Per cent	Number	Per cent
1[b]	20[c]	31	250 ♀	250	100.0	12	4.8
2	20	50	726 ♀	719	99.1	50	6.8
3	20	58	586 ♀	433	73.9	20	3.5
4	20	67	514 ♀	227	44.1	0	0.0
5	20	79	405 ♀	105	25.9	0	0.0
6	20	82	667 ♂	40	6.1	0	0.0
7	21[c]	58	755 ♀	143	18.9	5	0.6
8	21	62	394 ♀	60	15.1	0	0.0
9	23[d]	53	1,690 ♀	793	46.9	1	0.05
10	24[d]	64	592 ♀	387	65.3	2	0.3
11	24	84	1,103 ♀	688	62.3	11	0.9
			387 ♂	85	21.9	0	0.0
12	25[d]	31	1,292 ♀	1,182	91.4	11	0.8
			799[e] ♂	403	50.4	5	0.6
13	24	84	188[f] ♀	78	41.4	1	0 5
			340[g] ♀	272	80.0	20	5.8
			205[h] ♀	81	39.5	1	0.5
Totals	....	....	10,893	5,946	54.5	139	1.2

[a] Lots 1 to 12 were in the first field trial.   Lot No. 13 was in the second field trial.
[b] This lot consisted of 513 birds of which 250 were vaccinated and 263 left unvaccinated for controls.
[c] This vaccine undiluted contained 0.1 gram of tissue per cubic centimeter.
[d] This vaccine undiluted contained 0.25 gram of tissue per cubic centimeter.
[e] White Leghorn and Barred Plymouth Rocks from three to six months old.
[f] White Leghorns and Barred Plymouth Rock pullets four and one-half months old.
[g] White Leghorn pullets from six to seven months old.   Egg production was about 60 per cent at time of vaccination.
[h] Barred Plymouth Rock pullets from six to seven months old.   Egg production was about 60 per cent at the time of vaccination.

Discussion of Results.—As shown in table 11, a total of 10,893 pullets and cockerels from three to seven months old were vaccinated. Chicken-pox lesions developed at the point of vaccination on 5,946, or 54.5 per cent, of the birds, and about the head of 139, or 1.2 per cent, of the birds. In no case, however, did either the lesions at the point of vaccination or those about the head become extensive or in any way prove harmful. The lesions healed without treatment in every instance within 7 to 15 days after they were first observed.

Table 11 also shows that there was wide variation in the percentage of fowls in the different lots that developed lesions at vaccination point or about the head. The results obtained in lots 1 to 5 that were vaccinated with vaccine No. 20 indicate that the age of the vaccine was a factor responsible for such variation. This vaccine was administered to pullets when it was 31, 50, 58, 67, and 79 days old; and produced vaccination-point lesions on 100.0 per cent, 99.1 per cent, 73.9 per cent, 44.1 per cent, and 25.9 per cent of the fowls, respectively. The percentage of the fowls that developed lesions about the head was 4.8, 6.8, and 3.5, respectively, from the 31, 50, and 58-day-old vaccine and none from the 67 and 79-day-old vaccine.

Differences between vaccines of the same age with respect to the percentage of fowls upon which vaccination lesions were produced were also noted. For example, 58-day-old vaccine No. 20 caused lesions on 73.9 per cent of the fowls vaccinated, while but 18.9 per cent of the fowls vaccinated with vaccine No. 21 when it was the same age were so affected. Similar, though less marked, variations in the results were obtained with other vaccines administered when they were approximately the same age can be seen in table 11.

Variation with respect to the percentage of fowls with vaccination lesions also occurred in two instances in pullets and cockerels that received the same vaccine at approximately the same time. In the first instance, vaccination lesions developed on 25.9 per cent of the pullets that were vaccinated with 79-day-old vaccine No. 20 and on 6.1 per cent of cockerels vaccinated with the same vaccine when it was only three days older. In the second instance, vaccination lesions were produced on 62.3 per cent of the pullets and 21.9 per cent of the cockerels which received 84-day-old vaccine No. 24. That such marked differences between the percentage of pullets and cockerels that develop lesions following vaccination do not always occur, however, is illustrated by the results obtained with lot 13. In this case, vaccination of nearly an equal number of pullets and cockerels with the same vaccine resulted in vaccination-point lesions on nearly the same number of birds of each sex.

The results obtained with the flocks of 340 Leghorn pullets and 205 Barred Plymouth Rock pullets of lot 13 that were the same age showed that the same vaccine might affect different flocks of the same sex differently. In this case vaccination-point lesions developed on 80 per cent of the Leghorns and 39.5 per cent of the Barred Rocks, and head lesions developed on 5.8 per cent of the Leghorns and 0.5 per cent of the Barred Rocks. These flocks differed as regards breed but they were of the same age and were producing at the same rate.

The results of these field trials indicate that the vaccination of young fowls by subcutaneous injection of vaccine containing virulent chicken-pox virus is unlikely to produce *harmful* chicken-pox infection. Chicken-pox lesions may be produced, however, both at the point of vaccination and about the head. Such lesions are very limited in extent and may be expected to heal without treatment within 7 to 15 days. The number of fowls that will develop lesions at the point of vaccination is variable but in most instances relatively great and may include all of the birds vaccinated. The number that develop lesions about the head, however, may be expected to be relatively very small and in many instances such lesions will be entirely absent. The variation in the number of fowls that develop lesions after vaccination is to a considerable extent dependent upon the age of the vaccine at the time of administration. However, variations will occur in the effects of different lots of vaccine of the same age and in the effects of the same vaccine on different lots of birds of the same or opposite sex. It cannot be stated, therefore, that different lots of vaccine that are prepared in an identical manner, stored for the same length of time under the same conditions, and administered in the same dosage, will produce the same reaction in fowls vaccinated with them. Because of the differences in the behavior of apparently identical vaccines, it is impossible to predict the length of time that a vaccine will retain either its property of producing a visible reaction or its potency as an immunizing agent.

A visible reaction in the form of a lesion at the point of vaccination, while not absolutely essential to the production of immunity, usually indicates that the fowl will become immune and perhaps that the immunity will be more lasting than would be the case if no visible reaction occurred. To a certain extent, therefore, the percentage of birds of a flock that develop vaccination-point lesions may be considered as an index of the immunizing value or potency of the vaccine used.

Immunity to Chicken-pox.—During the winter and spring months after vaccination, no chicken-pox occurred in the flock except that which resulted directly from vaccination. Chicken-pox was exceedingly prevalent throughout the state during this time. Furthermore, as stated previously, on one farm chicken-pox had been continuously present for a year before the beginning of these vaccination field trials and on the other farm the disease had occurred during the five preceding winters. These facts suggest that the freedom of the vaccinated birds from chicken-pox resulted from protection afforded them by the vaccine. However, the lack of unvaccinated controls in the flocks makes it impossible to conclude that such was the case.

Evidence that the 250 birds of lot No. 1 of vaccinated fowls were immunized against chicken-pox during the second month after vaccination was furnished by the occurrence of chicken-pox among the 263 non-vaccinated controls that were in the same pen with those vaccinated (see footnote *b*, table 11). The vaccine was administered on April 30. Thirty-four days later the first case of chicken-pox was observed among the controls. Additional cases appeared during the next forty days until 114, or 43.3 per cent, of the controls became infected. All of the vaccinated birds remained free from the disease.

The prevalence of chicken-pox among the unvaccinated susceptible fowls that were allowed to associate with vaccinated fowls having vaccine-produced chicken-pox lesions was an expected and natural occurrence. However, it serves as a concrete illustration of the inadvisability of vaccinating any fowls on a poultry farm with vaccine containing virulent virus unless all of the fowls that are susceptible to chicken-pox are to be vaccinated.

Some of the vaccinated fowls were secured at irregular intervals after vaccination and tested for immunity. The tests consisted in severely scarifying approximately one square centimeter of the comb surface of each bird and applying a suspension in sterile saline of highly virulent chicken-pox virus to the scarified surface. Since birds for this purpose could be secured only as they were to be marketed, it was impossible to follow any definite scheme in making the tests. A total of 165 birds were tested for immunity at intervals after vaccination varying from 54 to 275 days. The data regarding the birds that were tested and the results obtained are summarized in table 12.

TABLE 12

RESULTS OF IMMUNITY TESTS OF FOWLS VACCINATED IN THE FIELD TRIALS OF VACCINE PREPARED FROM LESION TISSUE

Vaccine No.	Age of vaccine with which fowls were vaccinated (days)	Per cent of fowls that had developed vaccination-point lesions	Days after vaccination when tested	Fowls that were tested for immunity			Fowls that had developed vaccination-point lesions					Fowls that had not developed vaccination-point lesions				
				Total number tested	Number immune	Number not immune[a]	Number tested	Number immune	Fowls not immune[a] — Number	Number with slight lesions	Number with moderate lesions	Number tested	Number immune	Fowls not immune[a] — Total	Number with slight lesions	Number with moderate lesions
20	31	100.0	98	4	4	0	4	4	0	0	0	....	....	....	....	....
20	31	100.0	158	8	8	0	8	8	0	0	0	....	....	....	....	....
20	50	99.1	192–207	8	8	0	8	8	0	0	0	....	....	....	....	....
			274	5	1	4	5	1	4	2	2	....	....	....	....	....
25	31	91.4	189	5	3	2	5	3	2	2	0	....	....	....	....	....
25	31	91.4	54	24	18	6	22	16	6	6	0	2	2	....	....	....
20	84	73.9	140–149	5	5	0	5	5	0	0	0	....	....	....	....	....
20	58	73.9	266–275	5	2	3	5	2	3	1	2	2	2	0	1	0
24	64	65.3	88	10	9	1	5	5	0	0	0	5	4	1	1	0
24	58	65.3	178–182	6	2	4	2	2	0	0	0	4	0	4	4	0
24	84	62.3	67	10	10	0	5	5	0	0	0	5	5	0	0	0
23	53	46.9	152–161	5	4	1	1	1	0	1	0	4	3	1	1	0
23	53	46.9	110	25	3	22	8	0	8	3	5	17	3	14	7	7
20	67	44.1	196–205	10	9	1	5	5	0	0	0	5	4	1	0	0
20	57	44.1	274	4	4	0	0	0	0	0	0	4	0	4	4	0
20	57	18.9	257–266	10	5	5	5	5	0	0	0	5	1	4	0	1
20	57	18.9	172	5	1	4	0	0	0	1	0	5	1	4	4	0
21	58	18.9	150	5	4	6	5	0	3	0	1	....	....	3	0	1
			234	4	0	4	0	0	0	0	0	4	0	4	0	4
Totals				**165**	**100**	**65**	**97**	**68**	**29**	**16**	**13**	**68**	**32**	**36**	**23**	**13**

[a] The lesions on the fowls that were not immune in no case spread beyond the confines of the scarified area nor were otherwise pronounced. They were classified as slight or moderate.

Slight lesions consisted of the formation of a small amount of dry yellowish scab which at no time had the appearance of an active chicken-pox lesion. They were healed in 12 to 18 days after inoculation.

Moderate lesions began development like an active lesion. Drying occurred within a few days, however, and the lesions were entirely healed in 12 to 25 days after inoculation.

Non-vaccinated control fowls were inoculated with each lot of vaccinated birds. In all cases the controls developed pronounced lesions which were still active when the lesions on the vaccinated birds were entirely healed.

As shown in table 12, of the total of 165 fowls that were tested for immunity, 100, or 60.6 per cent, were immune to the infection and 65, or 39.3 per cent, were to some degree susceptible to the infection. Of the 65 fowls that were not entirely immune, 39, or 60 per cent, developed slight lesions and 26, or 40 per cent, developed moderate lesions. The control fowls which were inoculated with the same virus and at the same time as the vaccinated birds developed pronounced lesions that spread to cover an area from three to four times as large as that scarified and that were still active when the inoculation wounds or lesions on the combs of the vaccinated birds were entirely healed. It may be said, therefore, that all of the 165 vaccinated birds that were tested for immunity in from 54 to 275 days after vaccination were either immune or highly resistant to the artificial infection with chicken-pox virus.

The data in table 12 show that 97 of the 165 birds that were tested for immunity were from those that had developed lesions at the point of injection of the vaccine and that 68 were from those that had not developed vaccination-point lesions. Of the 97 birds that had developed vaccination lesions, 68, or 70.1 per cent, were immune; and of the 68 birds that had not developed vaccination-point lesions, 32, or 47 per cent, were immune.

To further demonstrate that the percentage of completely immune fowls was greater among those that had developed vaccination-point lesions than among those that had not developed vaccination-point lesions, the data are grouped in table 13 according to the percentage of fowls which developed vaccination-point lesions. But little difference with respect to the percentage of fowls that were completely immunized was found to exist between the lots of fowls of which 62.3, 73.9, 91.4, 99.1, or 100 per cent developed vaccination-point lesions and those of which 18.9, 25.9, 44.1, or 46.9 per cent developed vaccination-point lesions. Therefore, the fowls are grouped as those of which from 62.3 to 100 per cent (group 1) developed vaccination-point lesions and as those of which from 18.9 to 46.9 per cent (group 2) developed vaccination-point lesions. The data in table 13 are also arranged to show the variation in the percentage of fowls that were completely immunized with respect to the time after vaccination when the test for immunity is made. The number of birds is too small to permit consideration of each lot of birds with respect to the time between vaccination and the test for immunity; therefore, in table 13, grouping is made of those fowls which were tested for immunity within 207 days after vaccination and of those fowls which were tested in from 234 to 275 days after vaccination.

TABLE 13

THE RELATION OF THE PERCENTAGE OF FOWLS THAT DEVELOPED VACCINATION-
POINT LESIONS AND OF THE TIME BETWEEN VACCINATION AND THE
TEST FOR IMMUNITY TO THE PERCENTAGE OF FOWLS
THAT WERE COMPLETELY IMMUNIZED

Group No.	Per cent of fowls that developed vaccination-point lesions	Time after vaccination when tested for immunity *days*	Number of fowls	Number immune	Per cent immune	Fowls that had developed vaccination-point lesions			Fowls that had not developed vaccination-point lesions		
						Total number	Number immune	Per cent immune	Total number	Number immune	Per cent immune
1	62.3 to 100	54 to 207	81	67	82.7	61	53	86.8	20	14	70.0
		234 to 275	10	3	30.0	8	2	25.0	2	1	50 0
		54 to^a 275	91	70	76.9	69	55	79.7	22	15	67 2
2	18.9 to 46.9	54 to 207	65	29	44.6	28	13	46.4	37	16	43.2
		234 to 275	9	1	11.1	0	0	0	9	1	11.1
		54 to^b 275	74	30	40.5	28	13	46.4	46	17	36 9

ᵃ Includes all fowls of group 1.   ᵇ Includes all fowls of group 2.

In table 13, it is again shown that the percentage of fowls that
were completely immunized to chicken-pox was greater among those
which had developed vaccination-point lesions than among those which
had not. The percentage of the fowls developing vaccination-point
lesions that were immunized was greater in the group in which a
majority developed such lesions than in the group in which a minority
developed them. Table 13 also shows that the percentage of fowls
that were completely immunized was greater in the former group
than in the latter group, even among the fowls that did not develop
vaccination-point lesions. These results indicate that the percentage
of fowls that become completely immunized from vaccination among
those that either do or do not develop vaccination-point lesions will
be greater when 60 per cent or more of the vaccinated fowls develop
vaccination-point lesions than when less than 50 per cent of the
vaccinated fowls develop vaccination-point lesions. This applies par-
ticularly to the results with the fowls that were tested for immunity
within 207 days after vaccination. These comprised 146 of the 165
fowls, leaving but 19 fowls that were tested for immunity in from
234 to 275 days after vaccination. The percentage of these latter that
developed vaccination-point lesions and the percentage that was found
to be completely immunized were much less than was the case among
the fowls that were tested earlier. The number of birds, however, is

too small to permit accurate comparisons of results obtained with those obtained with birds among which a larger percentage developed vaccination-point lesions or which were tested for immunity sooner after vaccination.

In this discussion of the results of tests for the immunity of vaccinated birds to chicken-pox, particular attention has to be paid to the number of birds that were completely immunized. It should be remembered, however, that all of the fowls which were not completely immunized had a high degree of resistance to the infection irrespective of whether they had or had not developed vaccination-point lesions and of the time after vaccination when the tests for immunity were made. In the majority of cases, the degree of resistance amounted to nearly complete protection against the artificial infection with chicken-pox virus.

CONCLUSIONS

This paper presents the results of studies in the immunization of fowls against chicken-pox by the subcutaneous injection of vaccine prepared by mixing finely ground fresh tissue obtained from cockerels with pronounced comb infection of chicken-pox with a suitable liquid diluent. In these studies approximately one thousand birds that were kept under laboratory conditions and fourteen thousand birds on poultry farms have been utilized.

In the preparation of vaccine, the entire comb, or the lesion and sub-lesion epithelial tissue, either alone or with the addition of the liver, spleen, kidneys, and blood, was used. The lesion and sub-lesion epithelial tissue, alone, however, appeared to be the more satisfactory. As a diluent or vehicle and preservative for the tissue, either 0.5-percent phenolized physiologic salt solution or a mixture of equal parts of glycerine and 1.0-per-cent phenolized physiologic salt solution was used. The latter seemed to be preferable. The conclusions which follow are based upon the results of experiments with vaccines prepared from lesions and sub-lesion epithelial tissue and a mixture of equal parts of glycerine and 1.0-per-cent phenolized physiologic saline.

Such vaccine is capable of producing in fowls within 28 days after administration either complete immunity or a high degree of resistance to artificial infection with chicken-pox virus. The immunity or resistance has been shown to last for at least as long as 275 days. The data, however, are insufficient to permit conclusions regarding the percentage of vaccinated fowls that will remain completely immunized for such a long period after vaccination.

The immunizing value of vaccine has been shown to depend upon and to vary according to the amount and virulence of the virus it contains. No method has yet been devised, however, by means of which less than marked differences between the virus content of vaccines can be detected.

The results of the experiments have demonstrated that attenuation of the virus is not necessary to make the vaccine safe for use. However, slight attenuation does not injure the immunizing value of vaccine and may make more remote the possibility of harmful chicken-pox lesions resulting from subcutaneous injection. This may be accomplished by aging vaccine for a short period. Aging also serves to destroy contaminating bacteria. The length of time that vaccine may be aged without the virus becoming too much attenuated will vary according to the concentration of tissue, although variation in this respect also occurs in different vaccines with the same concentration of tissue. It is desirable, therefore, to use no more diluent in the preparation of vaccine than is necessary to facilitate the grinding process. In these experiments, sufficient diluent to make the concentration of tissue in vaccine 0.1 or 0.25 gram per cubic centimeter was found to be satisfactory. The length of time which the virus in vaccine of such concentration withstood aging without too great attenuation varied from 50 to 140 days. In vaccines containing 0.01 gram or less of tissue in a cubic centimeter, however, the virus was entirely destroyed in less than 40 days.

The amount of tissue in an immunizing dose of vaccine may vary within wide limits. The amount that was used most extensively in these experiments and which gave satisfactory results was 0.002 gram. To provide 0.002 gram of tissue in a dose of vaccine, the more concentrated preparation is diluted so that the stipulated amount is contained in one cubic centimeter. The dilution should be made just before administering. For the production of immunity to artificial infection in 28 days after vaccination, one dose of vaccine is as effective as two doses. No data has been obtained, however, regarding the comparative length of the immunity produced by one dose and that produced by two doses.

The subcutaneous injection of vaccine containing an abundance of virulent virus is usually followed by the development of a chicken-pox lesion at the point of injection. Such lesions do not spread to other parts of the body nor otherwise become harmful. When vaccine that is not more than 30 days old is used, vaccination-point lesions will be produced on nearly all of the fowls. When older vaccine is

used, the percentage of fowls which will develop vaccination-point lesions decreases as the age of the vaccine increases. Variations in respect to the percentage of fowls that develop such lesions, however, will occur between different vaccines of the same age and between different lots of fowls that are vaccinated with the same vaccine. Lesions of slight magnitude may develop also about the head of a small percentage of fowls that are vaccinated with vaccine that is not more than 30 days old but they are not apt to occur when older vaccine is used. The occurence of such lesions, however, is subject to variation under the same circumstances as vaccination-point lesions. The lesions may be expected to heal without treatment within from 7 to 15 days without harming the fowl. The largest number of fowls in a flock on which such lesions have occurred after vaccination is 6.8 per cent of 726 fowls.

The percentage of fowls that develop vaccination-point lesions is to some extent an index of the virulence or of the degree of attenuation of the virus in the vaccine. The development of lesions at the point of injection of vaccine is not essential to production of immunity. Fowls which develop these lesions, however, will usually become immunized. All fowls that receive vaccine in which the presence of virulent virus can be demonstrated become either immune or highly resistant to artificial infection with chicken-pox virus. The percentage of fowls that become completely immunized, however, will be greater among those that have developed vaccination-point lesions than among those that have not. Furthermore, the percentage of all fowls that become completely immunized after vaccination, irrespective of whether they do or do not develop vaccination-point lesions, will be greater in flocks in which 60 per cent or more of the fowls develop vaccination-point lesions than in flocks in which a smaller percentage of the fowls develop such lesions. The development of these lesions therefore may be regarded as a favorable vaccination reaction.

The vaccine may be administered to fowls in flocks in which an outbreak of chicken-pox exists without increasing the severity of the lesions in fowls already infected or hastening the spread of the infection among the healthy fowls. Vaccination of such flocks should result in control of the outbreak within 28 days.

Flocks of healthy young fowls from four to seven months old can be vaccinated for the purpose of immunizing them against subsequent infection without danger of inducing harmful chicken-pox infection among them. In such cases, however, all of the susceptible fowls on

the premises must be vaccinated. It would be unwise to use vaccine on a poultry farm on which chicken-pox had never existed unless it was so situated that chicken-pox virus was likely to be introduced on it at any time.

Although the data obtained in these experiments are not sufficient to prove definitely that when young fowls are vaccinated during the summer and fall they will be protected against chicken-pox infection during the following winter and spring, they nevertheless suggest that such may be the case.

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RELATION OF TUBER MATURITY AND OF STORAGE FACTORS TO POTATO DORMANCY

J. T. ROSA[1]

FOREWORD

Though the buds on a newly harvested potato tuber do not grow for sometime after the whole or a part of it is planted, even under conditions favorable for growth, they sprout rapidly after the tuber has been held in storage for a period of from a few weeks to several months. The time after harvesting during which these buds will not sprout, or do so very slowly, is called the dormant period. The causes of the inception of this condition are unknown. Schmid[17] and Appleman[3] suggested that its continuance is largely due to a lack of oxygen in the internal tissues. Appleman showed that when the periderm was removed, or when oxygen was introduced by other means, sprouting of dormant tubers was hastened.

Knowledge of the conditions causing dormancy, and of methods for shortening or "breaking" the dormant period, are of practical value. The growing season in California and in the southern states is long enough to produce two crops of potatoes yearly. To do this economically, it is necessary to use tubers produced by the early or spring crop, as seed for the late or fall crop. The period intervening between the two crops is so short, however, that the dormancy of the spring crop tubers often results in slow and irregular sprouting, if they are used for planting the fall crop. Another aspect of the dormancy problem is in connection with storage of table or seed potatoes, where it is desired to continue the dormant condition as long as possible.

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Work on potato dormancy was begun at Davis, California, in 1922. In the years since, much data have been accumulated. Many of the experiments, however, were of a preliminary nature, and have not been followed up intensively, because other methods of treatment seemed more promising. Some of the results obtained with chemical stimulants have been reported elsewhere by Rosa[15, 16] In this paper will be given the results of some of the experiments on certain phases of the problem, dealing with the behavior of potato tubers in relation to their maturity and their storage environment. No attempt is made to give results of the effect of the various treatments upon yield; the field experiments have not been extensive enough to give reliable data on this point. It may be assumed, however, that treatments which result in more rapid sprouting and in a higher percentage of germination, will increase the yield of both spring and fall crops, but especially of the latter, for which the growing period is usually cut short by frost.

MATERIALS AND METHODS

Two varieties, White Rose and Idaho Rural, have been used in most of the experiments. The first, which is the most important commercial variety in California, is also known here as Wisconsin, Wisconsin Pride, and Great Divide, and in the East as American Giant and Empire State. The second variety is little grown in California but is much used in the Pacific Northwest, where is its also known as Charles Downing and Earliest of All. A third variety, Irish Cobbler, was included in a few experiments. It has a long dormant period, which is difficult to break, while the White Rose and Idaho Rural varieties have short, easily broken dormant periods. Tubers grown at the University Farm, both in spring and fall crops, have been used. Experimental plantings have been made in soil in the field, and in sand in coldframes and in the greenhouse. The first method represents ordinary practical conditions, while the second and third allow better control of moisture and temperature. The tubers or seed pieces, which are hereafter referred to as "sets," were planted about 3 inches deep. Cut sets averaging about 30 grams in weight were used in all tests, unless otherwise specified. Counts were made every three or four days of the sprouts as they appeared above the surface of the soil.

In the calculation of results, the arrival of the sprouts above ground is referred to as emergence. The best index of the speed of sprouting is the average number of days required after planting for

the sprouts to reach the surface of the soil.   This numerical index is
low when sprouting is rapid and high when sprouting is slow.   The
proportion of the sets planted, which produced plants, is referred to
as "per cent stand."

RELATION OF TUBER MATURITY TO DORMANCY

In 1925, samples were dug at 10-day intervals from the spring
crop of White Rose and Idaho Rural, in 1926 of Idaho Rural, and in
1927 of White Rose, Idaho Rural, and Irish Cobbler.   The first sample
was dug as soon as tubers large enough for cut sets were available.
At this harvest, as well as at the second, the plants were fully green
and plants and tubers were growing rapidly.   At the third and fourth
harvests, the plants were showing signs of maturity, with some of the
leaves dead.   At the fifth harvest (in 1926 and 1927) the tops were
completely dead.   The tubers increased in size and in maturity during
this period.   Only at the last harvest in each year was the skin
suberized sufficiently so that it did not rub off easily.   Each sample
was divided, one part being placed in cold storage at 4° Centigrade,
the other in a cellar at 23° C, except in 1927, when cellar storage alone
was employed.   The samples were held until July 26 in 1925, July 14
in 1926, and July 27 in 1927, when they were removed from storage,
cut and planted.   Thus in 1925 the storage period for the first sample
was 57 days and for the last, 26 days.   In 1926 and 1927 the storage
period ranged from 55 down to 15 days.   No consistent differences in
results were apparent between the halves of the samples stored at 4°
and at 23° C, hence the data have been combined, as shown in table 1.

With the White Rose variety in 1925, the percentage of stand
seemed to be slightly higher in the samples dug early, but in the other
ten series it was higher in the lots harvested late.   This was especially
true in 1927, with all three varieties, both under coldframe and field
conditions.   Those sets which did not produce plants, decayed.   This
decay, which occurs within a few days after planting, is probably
caused by fungi which invade the wounded surface of the cut sets.
The early harvested, immature tubers in these experiments were
rather small.   In other tests, it has been noted that where large
immature tubers were used, the amount of seed piece decayed was
much greater.   There is some evidence that cut sets from tubers fully
ripened before harvest, are less subject to decay after planting.

With regard to the rate of sprouting, the twelve series of tests
were all in agreement.   The average number of days required for the

TABLE 1

GROWTH-RESPONSE OF TUBERS HARVESTED AT DIFFERENT STAGES OF MATURITY

		Coldframe planting				Field planting		
		Number of sets planted	Per cent stand	Average number of days to emerge	Average number of stems per set	Number of sets planted	Per cent stand	Average number of days to emerge
White Rose, 1925								
Harvested	May 30	60	76.7	37 2	1 15	167	67.7	44.3
	June 10.....	60	78.3	33 7	1.40	162	63.0	43.8
	June 20	119	73 6	30.0	1 22	172	58.7	35.7
	July 1....	60	63.4	25 4	1 58	157	58.6	29.7
Idaho Rural, 1925								
Harvested	May 30	59	78.0	47.1	1.13	166	66.2	55.7
	June 10 .. .	60	79 3	34.9	1 26	145	61 5	• 49.6
	June 20	90	80 0	35.6	1.16	172	59.2	39.0
	July 1... .	60	73 1	37.5	1.33	149	76 5	39.2
Idaho Rural, 1926								
Harvested	May 20 .	60	81 7	37.6	1 10	215	45 0	58 5
	May 30 . .	62	81.9	37 4	1 20	213	45 2	54 8
	June 9	80	90 3	34 1	1 35	207	53 0	48 7
	June 19 .	333	81 3	33 6	1 36	716	63 3	46 1
	June 29	60	91.6	28 3	1 67	183	64 5	40 4
White Rose, 1927								
Harvested	June 2 . .	30	40 0	37 5	1 17	117	62 4	49 5
	June 12 .	20	3 0	. .	.	105	48 6	46 1
	June 22	20	15 0	37 7	1 00	100	28 0	41 6
	July 2	130	72 3	33 5	1 28	577	61 7	34 0
	July 12	30	80 0	25 0	1 46	. 120	71 6	36.9
Idaho Rural, 1927								
Harvested	June 2 ...	30	13 3	38.5	1 00	114	44 7	53 5
	June 12. .	20		.		100	42 0	49.0
	June 22 .	20	30 0	35 2	1 17	112	39 3	42.4
	July 2 .	90	57 7	35 6	1 15	554	56 5	37.7
	July 30	30	66 6	32.1	1 05	120	74 1	45 8
Irish Cobbler, 1927								
Harvested	May 23.... . . .	30	26 6	43 4	1 00	100	46 0	48.5
	June 2....	30	43 3	43 8	1 31	114	64 0	48.2
	June 12	20	25 0 .	36 0	1 20	119	53.7	41.0
	June 22	20	65.0	38 8	1.15	115	60 8	39.6
	July 2....	60	80 0	37.2	1.25	120	78.6	36.2

sprouts to emerge was greatest in the samples harvested earliest, and decreased regularly with the period of harvesting, the time required being shortest in the samples dug most mature. This is somewhat surprising, as the samples dug early had a much longer period in storage before planting. These results probably explain why fall crop potatoes are slow in sprouting when used as seed for the spring crop, a fact noted previously by Rosa[14] and by Martin *et al.*[7] Fall crop

potatoes are harvested immature, as the tops are usually green when killed by frost.   Similar results have recently been published by Kolterman,[5] in Germany.   He states that the earlier a tuber is separated from its plant, the more time it needs to ripen and to germinate.

The more rapid sprouting of tubers harvested mature in these experiments are not to be considered as opposing the results of Mueller and Molz[8] and of many other investigators, who have found more vigorous plant growth and higher yields with seed tubers harvested immature. In experiments of the latter class, the tubers were stored over winter, ample time being allowed for even immature tubers to reach the end of the dormant period.

The average number of stems arising from each set increased with the maturity of the tubers.   This point is of practical importance, for the yield increases with the number of stems to the plant, within limits.

DURATION OF THE DORMANT PERIOD

Is the dormant period of definite duration, or is dormancy dissipated gradually?   Since the response of tubers harvested immature and fully matured is different, it has to be determined separately on each class of tubers.   Samples were harvested July 30 from an April 15 planting of White Rose and Idaho Rural, the plants being almost completely dead at this time, and from the fall crops of the same varieties a few days after the green plants were frosted in November.   The tubers in the latter case were nearly full grown but were immature and the skin was easily rubbed off.   The tubers were all stored at 20 to 23° C and plantings made at intervals in the greenhouse.   The time for sprout emergence for different plantings is shown in table 2.

TABLE 2

RATE OF SPROUTING OF MATURE AND IMMATURE TUBERS PLANTED AT DIFFERENT PERIODS AFTER HARVEST

Number of days after harvest, planted	Mature tubers		Number of days after harvest, planted	Immature tubers	
	Average number of days to emerge			Average number of days to emerge	
	White Rose	Idaho Rural		White Rose	Idaho Rural
6	55.9	77.3	10	84.8	82.7
15	38.2	60.8	15	84 2	74.2
28	20.0	38.4	22	73.8	70.8
39	18.2	30.2	33	43.0	38.4
55	21.2	29.7	43	36.0	32.3
....	....	....	66	29.8	37.5
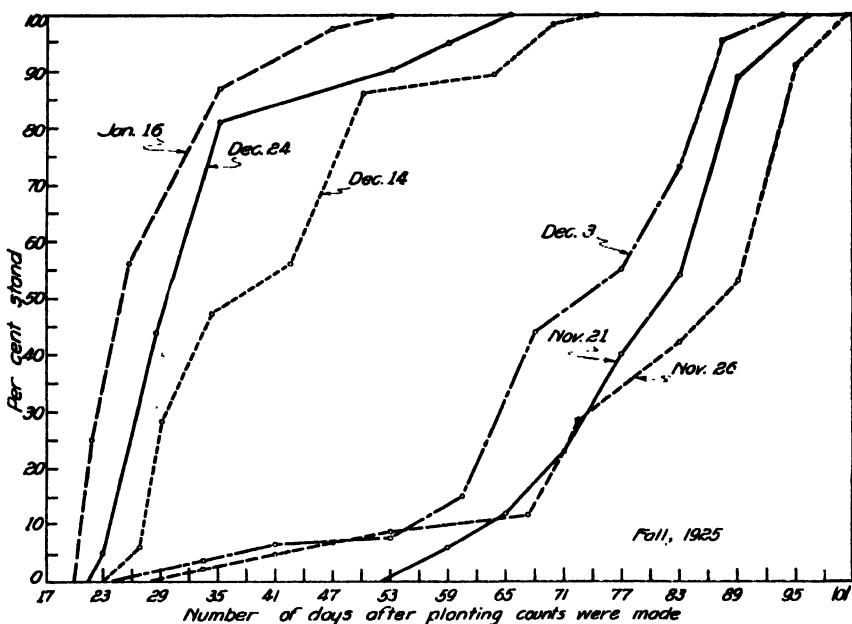
Fig. 1. Rate of sprouting of White Rose potatoes planted at different
dates after harvest. Harvested immature, Nov. 11, 1925.
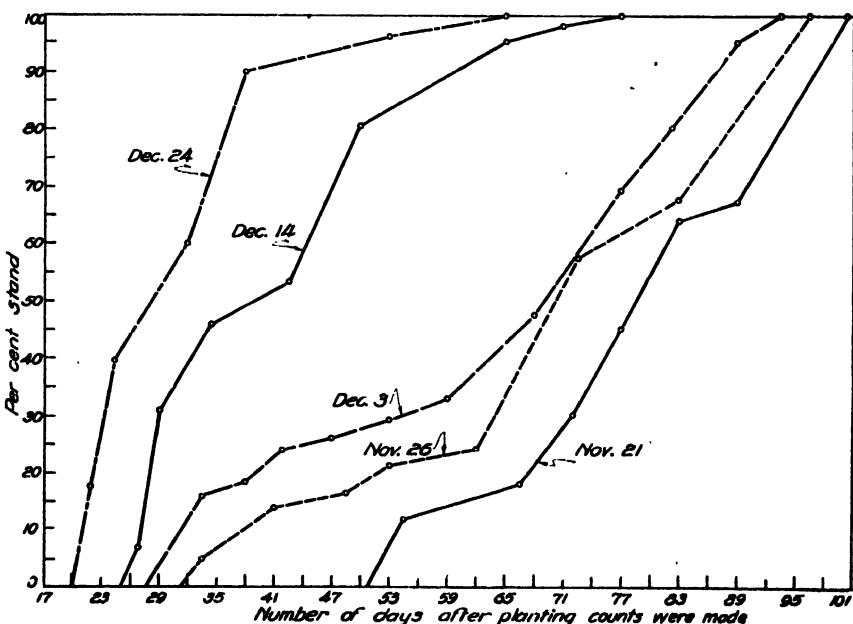
Fig. 2. Rate of sprouting of Idaho Rural potatoes, planted at different
dates after harvest. Harvested immature, Nov. 11, 1925.

Although the planting intervals for the two classes of tubers do not correspond exactly, it is easily seen in table 2 that the mature tubers reach a condition where they sprout rapidly, sooner than the immature ones. Thus, mature White Rose tubers may be said to be approaching the end of their dormant period at 28 days, and mature Idaho Rural at 39 days, after harvest. On the other hand, immature tubers of both varieties were still somewhat dormant at the end of 66 days. Emergence from the dormant condition is not abrupt; it is a gradual transition. The rates of sprouting for the immature tubers planted at successive dates are shown in figures 1 and 2. The graphs show that the longer tubers are held after harvest, the sooner do they sprout when planted; furthermore, the later planted tubers all sprout within a relatively short space of time, whereas the sprouting is irregular with tubers planted while still dormant.

It may be concluded that, from a practical point of view, spring crop potatoes to be used as seed for the fall crop should be allowed to become as mature as possible before harvesting, even though the subsequent storage period be very short. Also, it is indicated that when tubers harvested immature are used as seed, the need for the use of stimulants to hasten sprouting is greater than in the case of fully matured ones. Furthermore, immature tubers may be expected to remain unsprouted longer, when stored at ordinary temperatures, than mature ones.

RELATION OF STORAGE TEMPERATURE TO DORMANCY

It has been believed generally that placing dormant potatoes in cold storage for a period would result in growth-releasal upon their removal to temperatures suitable for growth. Müller-Thurgau[9] stated that storage of tubers at 0° C shortened the dormant period. His chief experiment involved 10 tubers harvested July 28 and held at 0° C for 40 days, then planted at 20° C. Sprouts formed about three weeks later. In a second test, tubers harvested August 28 were held at 20° C for 10 days, then at 0° C for 16 days longer. The tubers were then planted at 20° C, and formed sprouts within 18 days, a total period of 44 days after harvesting. No control or check lot held at ordinary temperature is mentioned in his report on either experiment. Newton,[11] working at the California Experiment Station, reported that immature tubers stored at 5° C sprouted slightly more rapidly than similar tubers stored at 20° C. His experimental lots, while very small (ten sets per culture), still gave consistent differ-

ences in several plantings made at successive intervals. Stuart[19] also writes: "The effect of the low temperature on the new potato is to shorten the rest period and hasten sprouting."

To test this hypothesis, samples dug June 20 and July 1, 1925, from nearly mature plants were placed in cold storage at 1° C and in a cellar at 23° C. The storage period was 36 days for the June 20 and 26 days for the July 1 samples. The tubers were large and their skin was moderately suberized. They were cut and planted July 26. The plantings were replicated in most cases two or more times. The results of both field and coldframe plantings are given in table 3, the data for samples stored for 36 days and 26 days being combined.

TABLE 3

RELATION OF STORAGE TEMPERATURE TO SPROUTING OF TUBERS HARVESTED NEARLY MATURE, 1925

Variety	Storage temperature	Coldframe planting			Field planting		
		Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose.......	1°C	89	68.0	28.5	172	52.1	35.0
White Rose........	23°C	120	70.0	26.5	157	67.8	33.3
Idaho Rural.....	1°C	60	83.3	36.9	180	65.0	43.0
Idaho Rural........	23₀C	120	74.2	36.2	156	71.0	37.1
Irish Cobbler......	1°C	30	60.0	57.3	131	· 52.7	51.3
Irish Cobbler.......	23°C	30	86.6	44.8	122 ·	62.3	39.0

In five of the six comparisons possible in table 3, the cellar-stored samples gave a higher percentage of stand than the cold-storage ones. In every case, the tubers stored at the higher temperature sprouted somewhat more rapidly than those from cold storage, though in several cases the difference in average number of days to emerge is hardly significant. This result is contrary to the general belief on this point, and contrary to the results of some other investigators. However, the results given here are borne out by the results of most of the later experiments.

In 1926, samples were harvested June 19 from nearly mature plants of two varieties, and were stored at 4°, 8°, 23°, and 30° C. They were cut and planted July 14, after only 25 days in storage. The results are given in table 4.

Storing the Idaho Rural variety at different temperatures seemed to result in no consistent difference in percentage of stand, but with·

the White Rose, which was less mature at harvest time, the lots at the two higher temperatures gave a higher percentage of stand than did those in cold storage. There was little difference in time of emergence of the samples stored at 4°, 8°, and 23° C, though the field plantings indicate more rapid sprouting of tubers that had been stored at the higher temperatures. But in all cases those which had been stored at 30° C sprouted much more rapidly than those at lower temperatures. The 30° C storage was in a basement room, heated with electric light globes, the air being constantly stirred by a fan. As the air was drier than that of any of the other storage rooms, the moisture conditions seemed to be the less favorable for sprouting.

TABLE 4

RELATION OF STORAGE TEMPERATURE TO SPROUTING OF POTATOES, 1926

Variety	Storage temperature	Coldframe planting			Field planting		
		Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
Idaho Rural.	4°C	90	89 2	32.1	208	69 2	42 5
	8°C	55	78 6	33 5	109	56.7	38 6
	23°C	161	76 8	34.6	399	61 6	31.0
	30°C	60	86 6	26.5	106	65.1	31.0
White Rose .	4°C	30	63 2	32 3	78	47.5	42.2
	8°C	60	52.6	32 7	106	49 1	39.8
	23°C	60	74 9	34 3	57	58 0	32 1
	30°C	30	86.6	25.1	108	52.2	27.8

In 1927, three varieties were employed for the temperature experiments. The White Rose and Idaho Rural were dug July 6, and stored at the different temperatures from July 8 to July 27, a period of 19 days. The plants were nearly dead at harvest time, and the tubers were fairly well suberized when they were placed in storage. The Irish Cobbler potatoes were fully matured; they were stored for a period of 25 days. The results are given in table 5.

As in previous experiments tubers stored at 22° and 30° gave in most cases a higher per cent of stand than those held in cold storage. This was especially marked in the field planting.

The lot stored at —0° C were in a room supposedly constant for 0° C, but the temperature fell below this point. The exact temperature range for this room is unknown; however some of the tubers stored there were frozen and decomposed promptly upon removal from storage. Over half the tubers did not freeze but remained

sound, though they must have been subject to prolonged under-cooling at below-freezing temperatures. Comparing the germination of these under-cooled tubers to those stored at higher temperatures, it appears that they sprouted somewhat more rapidly. Wright and Peacock[20] report somewhat similar results with under-cooled tubers.

Comparing the lots stored at 4° to those stored at 22° C, it is again seen that sprouting is somewhat slower in tubers that have been stored at the lower temperature. This is true for both coldframe and field plantings of the three varieties used in this experiment. As in the tests of the previous year, however, the difference in sprouting

TABLE 5

RELATION OF STORAGE TEMPERATURE TO SPROUTING OF POTATOES, 1927

Variety	Storage temperature	Coldframe planting			Field planting		
		Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose	−0°C	30	90.0	32.5	102	75.4	31.7
	4°C	65	80.4	37.7	118	36.5	34.3
	8°C	30	76.6	34.8	120	40.8	43.2
	22°C	130	72.3	33.6	577	61.7	34.0
	30°C	60	90.0	22.3	238	63.3	24.1
Idaho Rural	4°C	89	86.0	44.3	217	49.6	43.2
	22°C	90	57.7	35.6	554	56.5	37.7
	30°C	60	71.6	24.3	200	63.5	28.9
Irish Cobbler	4°C	29	72.1	42.0	120	42.5	45.0
	22°C	60	80.0	37.2	236	78.6	36.2
	30°C	30	63.3	35.3	120	52.0	35.0

rate between 22° and the lower temperatures are slight, compared to the large difference between 22° and 30°. Tubers that had been stored at the latter temperature sprouted much more rapidly. Figure 3 shows the difference in number of plants above ground, 25 days after planting, in experiments with tubers stored at 4° and at 30° C.

It may be suggested that this striking effect of high temperature upon dormant tubers be due to some direct physical effect, beside the increase in the normal growth rate of the bud primordia and to the increased rate of chemical reactions in the cells associated with higher temperatures. In this connection, it may be noted that Müller-Thurgau and Schneider-Orelli[10] found that exposures of tubers to temperatures of 38°, 40° and 42° C for four to eight hours resulted in an increased respiration rate after their removal to a lower temperature. This indicates an increased oxygen intake by the tubers

after exposure to high temperature, which may be important in con-
nection with increased growth rate. These writers concluded that the
effect of heat was fundamentally a weakening of the protoplasm, like
that ensuing normally as the age of the tuber increases. Appleman[a]
found that heating potatoes at 40° C for 8 hours in a moist chamber
hastened sprouting, though the same treatment in a dry chamber had
less effect. Ajrekar and Ranadive[1] found that sound tubers of some
varieties could be kept for 9 days at temperatures as high as 42° C,
while others developed black heart at this temperature.
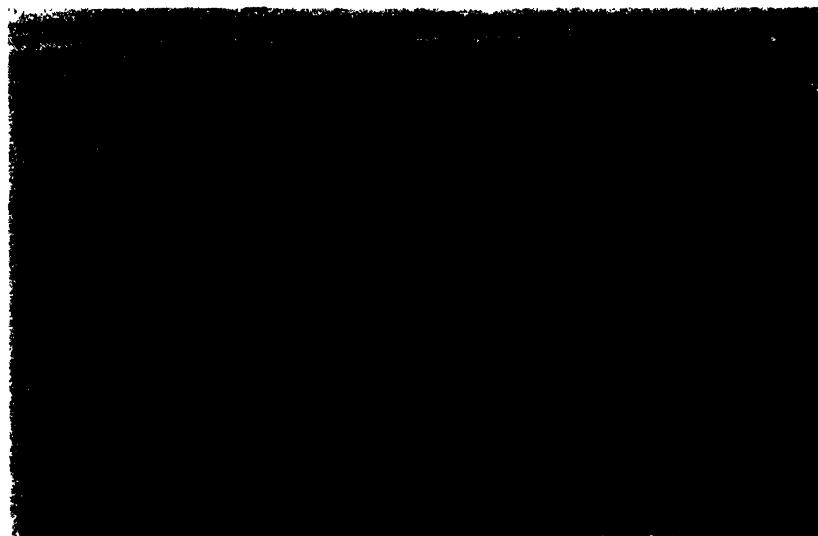
Fig. 3. Potato plants in coldframe, 25 days after planting, Aug. 21, 1927.
The four rows at the right are from tubers stored at 30° C. At the left are
four rows from tubers stored at 4° C.

Further evidence of the effect of storage temperature upon
dormancy was obtained in another experiment during the winter of
1925. Immature tubers that were large enough to cut into four or
more sets of about one ounce each were used. These were the product of
the fall crop, the plants of which had been frosted on November 5 and
the tubers harvested a week thereafter. They were stored in rooms
at 4°, 20°–22° and 27°–30° C. No sprouts appeared on the tubers
during storage at 4°; in fact, this temperature is somewhat below the
minimum for vegetative growth of the potato. At 20° to 22°, sprouts
appeared on the apical end of the tubers about the middle of January,
and at 27° to 30° late in December. Samples were removed and
planted in the greenhouse at intervals through the winter. Forty

sets were planted in each test, and a 100 per cent stand was obtained in all cases. The time for emergence of the different plantings is shown in table 6.

After 10 days in storage, the time for sprouting was the same in tubers from the three temperatures, but after 15 days there was a slight tendency to more rapid sprouting in samples from the higher temperature. This became marked at the 22-day and later periods. However, when the 66-day period was reached, there was again rather little difference in the rate of sprouting, for by this time even the tubers at 4° probably were near the end of their dormant period. It

TABLE 6

RATE OF SPROUTING OF IMMATURE WHITE ROSE POTATOES, AFTER STORAGE AT
DIFFERENT TEMPERATURES

Planting date	Average number of days to emerge		
	Stored at 4°C	Stored at 20°-22°C	Stored at 27°-30°C
Nov. 21, 10 days after harvest.	85.0	84.8	85.9
Nov. 26, 15 days after harvest.................	88.7	84.2	78.0
Dec. 3, 22 days after harvest	81.5	73.8	60 6
Dec. 14, 33 days after harvest	66.0	43.0	No test
Dec. 24, 43 days after harvest	41 0	36.0	No test
Jan. 16, 66 days after harvest	30.9	29.8	23.5

seems evident that tubers emerge from dormancy more slowly when stored at low temperatures. The differences in the time of sprout emergence as affected by temperature of the previous storage of the tubers, is shown graphically in figure 4, for the December 3 plantings. The maximum differences would probably be shown in the December 14 planting, 33 days after harvest, were that series complete. It should be remembered that the tubers used were harvested immature and are therefore not comparable to other tests, in which nearly mature tubers were used.

RELATION OF STORAGE HUMIDITY TO DORMANCY

It was suspected that the rather striking results secured in the temperature experiments might be due, at least in part, to the varying humidity of the storage rooms maintained at different temperatures. Appleman[3] showed that placing immature non-suberized tubers under moist conditions tended to prevent suberization and to promote earlier growth. As cold storage rooms often have a higher relative humidity than those at higher temperatures, the humidity factor may have been the cause of conflicting results of other investigators.

To determine the probable effect of the humidity factor, experiments were carried out in 1927 with two varieties at three temperaturse. Table 7 gives the results on large, nearly mature, well suberized tubers. Those samples designated in the table as "dry" are the same as those previously referred to in the temperature experiment. They were simply sacked and placed on shelves in the different temperature
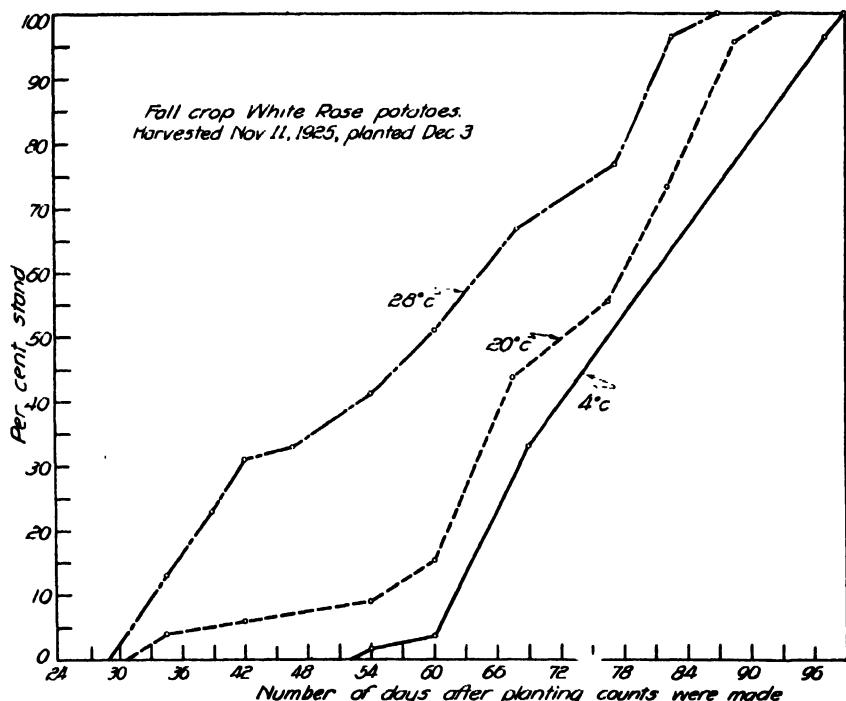
Fig. 4.   Rate of sprouting of potatoes harvested Nov. 11 and planted Dec. 3, after storage at different temperatures.

chambers. The relative humidity of the air in these chambers was approximately 75 at 4°, 55 at 22°, and 60 at 30°. The samples referred to as "moist" were moistened, placed in boxes, and covered with moist sawdust. This maintained an approximately saturated atmosphere around the tubers continuously, yet with free access of air to the tubers. Different rooms were used for the dry and the moist experiments at 30°, but neither room deviated over one-half of a degree from this temperature.

Table 7 shows that at 4° and at 30°, the humidity around the potato is without effect upon the subsequent rate of sprouting; those stored under both dry and moist conditions sprouted at approximately the same rate, except the field planting of the Idaho Rural, in which

the moist sample sprouted more rapidly. There were many well developed sprouts on the tubers of this lot at the time they were removed from storage. However, at the intermediate temperature, 22° C, the moist samples in every case sprouted much more rapidly than the dry or check lots. Apparently, humidity has an important effect upon dormancy of the tuber at ordinary temperatures. Besides inhibiting further suberization of the skin, it may be that the moisture also renders the skin more permeable. These effects, however, do little

TABLE 7

EFFECT OF HUMIDITY DURING STORAGE UPON SUBSEQUENT SPROUTING OF POTATO TUBERS, SUMMER OF 1927. STORAGE PERIOD 19 DAYS

Variety	Storage temperature degrees Centigrade	Coldframe planting			Field planting		
		Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose	4° dry	65	80.4	37.7	118	36.5	34.3
	4° moist	30	100.0	41.8	120	79.0	33.4
	22° dry	120	72.3	33.6	577	61.7	34.0
	22° moist	30	90.0	25.6	120	84 2	23.9
	30° dry	60	90.0	22.3	238	63.3	24.1
	30° moist	30	100.0	22.3	117	60.7	25.7
Idaho Rural . . .	4° dry	59	83.2	44.0	217	49.6	43.2
	4° wet	30	86.6	43.7	119	72.2	37.9
	22° dry	90	57.7	35.6	554	. 56.5	37.9
	22° wet	30	93.3	22.8	118	80.5	29.9
	30° dry	60	71.6	24.3	200	63.5	28.4
	30° wet	30	86.6	24.7	107	80.3	22.9

to abbreviate dormancy at 4° C, due to the limitation of the low temperature, and at 30° the effect of the moisture is not generally additive, because the high temperature itself brings about the most rapid termination of the dormant condition that is possible.

In almost every case, a higher per cent stand was obtained from the seed which had been stored under moist conditions. Just why the cut sets of the moist-stored tubers escaped decay to such an extent is not clear, but the fact is of much practical importance.

CHANGES IN THE TUBER DURING THE DORMANT PERIOD

. No significant difference in the chemical composition of dormant and non-dormant potato tubers has yet been established. Appleman[3] showed that the accumulation of sugar, which occurs when tubers are stored for a few weeks at temperature near 0° C, had no causal

Fig. 5. Vegetative sprouts arising from young partly grown tubers, while still attached to the mother plant. British Queen variety, Davis, California, June, 1924.

relation to growth releasal. Neither were there significant changes in the form of the nitrogen and phosphorous compounds during the dormant period. The same worker found that active diastase and invertase were present at all stages but did not increase until sprouting began. Oxidase and catalase activity also were found to be greater at the end of the dormant period.

Moreover, while Appleman and Miller[4] found that tubers harvested at different stages of maturity differed in composition when harvested, the composition of all was about the same at the end of the

dormant period. These workers paid especial attention to the various nitrogen fractions. They found that the total non-protein nitrogen, the amide and amino nitrogen increased progressively in tubers harvested at different stages of maturity. Accordingly they consider protein hydrolysis to be one of the important changes in the ripening
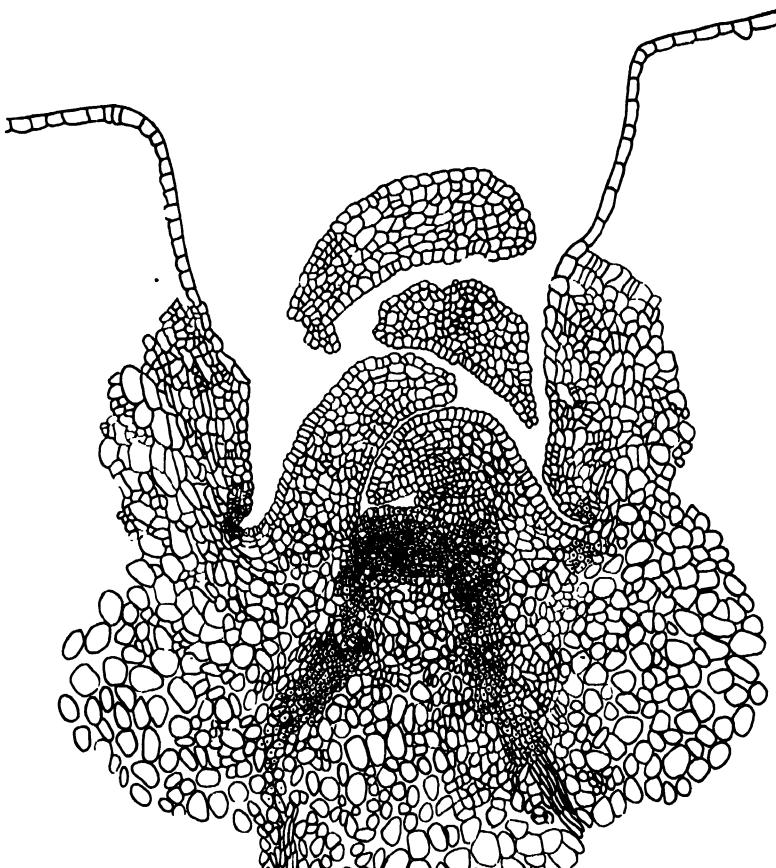
Fig. 6. Section through an eye of a partly grown tuber, weighing 91 grams, harvested May 31, 1924. The meristem of the sprout is seen beneath overlapping bracts at the base of the eye depression.

of the tuber. While these nitrogenous constituents tended to come to about the same value in immature and mature tuber after four months' storage for the former and two months for the latter, this after-ripening process probably is incomplete in tubers planted in less than two months after harvest, as was the case in the writer's experiments. Newton[11] considered that the ending of the dormant period depends in part upon the activity of the proteolytic enzymes and the enzymes which convert amino to amide nitrogen.

The dormant period of potato tubers probably is not an essential part of the life cycle. Some varieties of potatoes, when grown under conditions of high temperature and abundant moisture, may form vegetative sprouts from partly grown tubers. Figure 5 shows a case of this kind in the British Queen variety. Kolterman[5] reports a similar development in Germany, following an unusual drought. Dormancy must depend upon physical and chemical conditions within the tuber.
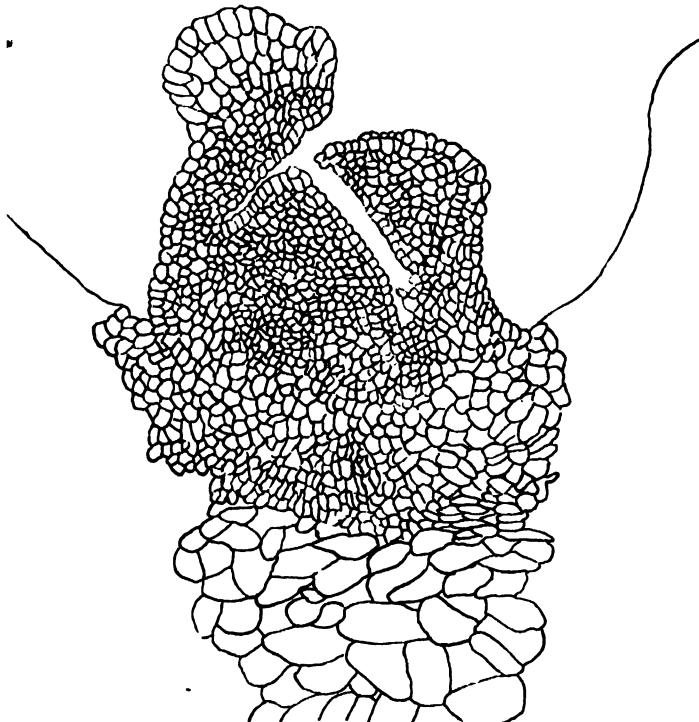
Fig. 7. Section through the eye of a large, immature White Rose tuber, just after harvest, Nov. 12, 1925. The central sprout, with its attendant bracts, is raised somewhat above the base of the eye depression.

The condition of the bud primordia in tubers at different stages of maturity and at different times in the dormant period has been investigated. Figure 6 is a section through an eye on the middle portion of a young White Rose tuber harvested May 30, 1925, and weighing 91 grams. While the bud primordia are present even in such very young tubers, sprout development has not commenced and the meristematic region is still deeply embedded in the tissue surrounding the eye. Smaller meristematic regions are visible on both sides of the bracts which envelop the central meristem. If the central

sprout is destroyed, other sprouts may be developed from the former. Figure 7 shows the apical eye of a large tuber, weighing 350 grams, harvested when nearly full grown but still immature. The sprout has already begun to form, though it is partly enclosed by the incurved bracts on each side. As the White Rose variety shows strong apical dominance, the apical sprout is much further developed than any
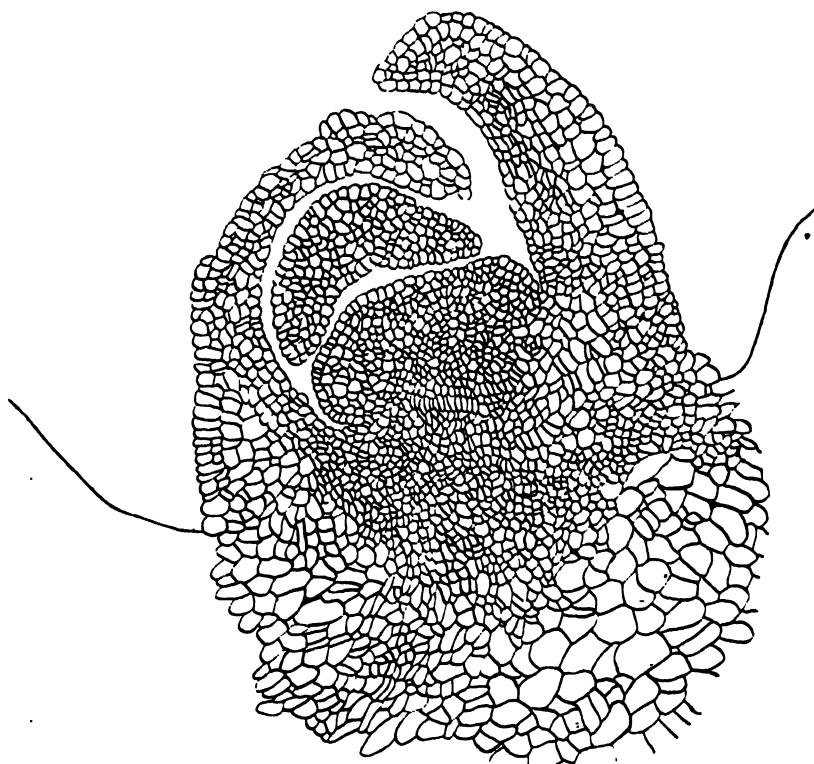
Fig. 8. Section through an eye of a large tuber similar to that shown in figure 7, 32 days after harvest. The young sprout shows further development in this interval. The storage was at 20° C.

other on the tuber. Figure 8 shows the apical eye of a tuber from the same lot as figure 7, but kept in storage at 20°–22° C for 32 days after harvesting. Further development of the sprout during this interval is shown. Figure 9 shows the sprout from an apical eye of a tuber from the same lot as that shown in figures 7 and 8, but kept in storage for 70 days after harvest. At this time, well developed sprouts were visible. These sections show that the bud primordia develop considerably during the late stages of tuber growth, and that some development of the sprout proceeds even during the so-called dormant
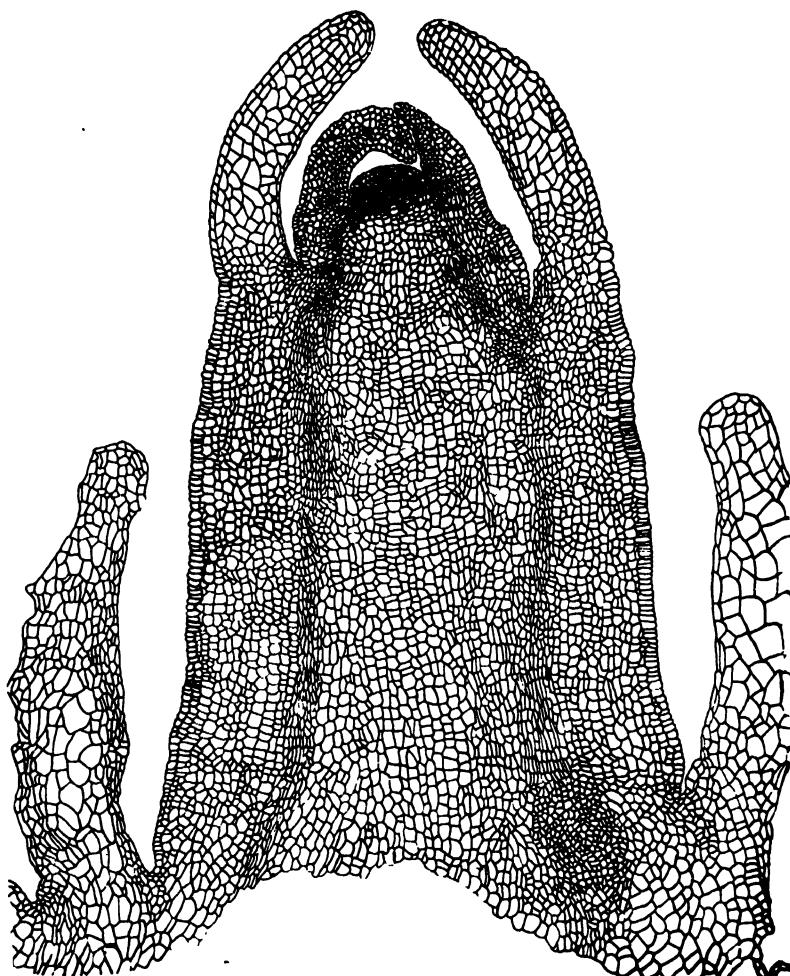
Fig. 9. Section through the sprout from an apical eye of a large tuber 70 days after harvest, and several days after planting. Elongation of the sprout becomes rapid at this stage.

period, if the tubers are stored at temperatures favorable for growth. The rate of sprout development increases toward the end of the dormant period.

VARIETAL DIFFERENCES IN DORMANCY

It is known that some varieties, such as McCormick, Bodega Red, and Irish Cobbler, remain dormant longer than others. Müller-Thurgau observed that the American variety, Early Rose, had a short dormant period when grown in Germany. Wilson Popenoe, of the U. S. Department of Agriculture, found a yellow-fleshed variety in.

Peru which was said to have no dormant period. Probably some of the differences observed in length of dormant period were due to differences in the stage of maturity at which the tubers were dug. Differences between the tubers within a variety may be due to the depth of the eyes. Deep-eyed tubers have a longer dormant period than shallow-eyed ones, probably because the bud primordia are so located in the former that the oxygen supply is less adequate for growth. The spindle-tuber disease has been reported to delay sprouting, but mosaic and other virus diseases have not been found to affect the dormancy of the tuber.

Several varieties grown in a spring crop (1924) were harvested June 22, when they were approaching maturity, though the tops were only partly dead. After one month's storage at 22° C, the tubers were cut and planted in the field, each plot consisting of 170 sets. The final count of the plants was made on October 13. Table 8 gives a summary of the results.

TABLE 8

VARIETAL DIFFERENCES IN THE RATE OF SPROUTING OF DORMANT POTATOES

Variety	Number of plots	Per cent stand	Average number of days to emerge
White Rose	15	62	39.0
Early Rose	1	55	39.4
Green Mountain	1	47	42.5
Idaho Rural	8	34	50.6
American Wonder	4	60	51.0
Bliss Triumph	1	32	55.0
Irish Cobbler	3	38	56.5
Garnet Chili	1	52	56.6

It appears that the White Rose and Early Rose varieties have the shortest dormant period and are the best suited, of those tested, to the practice of growing two crops a year. Thirty years ago, the Early Rose was much used in this way in the South Atlantic states, but the practice ceased when the Irish Cobbler became the leading commercial variety in that section. The longer natural dormant period in the latter variety probably made it difficult to grow two crops a year. The difference in length of dormant period noted here between Green Mountain and Irish Cobbler is in agreement with the results of Martin *et al.*,[7] who compared mature Maine-grown seed of these varieties with immature fall crop tubers from New Jersey. In the East Indies, Paravinci[12] reported that the Dutch varieties used there required a storage period of 100 days between harvest and planting in order to start growing promptly when planted.

SEED PIECE DECAY

Premature decay of the sets has been the chief cause of poor stands in potatoes planted during the summer at Davis. This is especially true of the field plantings. This decay is probably caused by fungi which invade the wounded cells of the cut surface. Tubers planted whole are much less subject to decay. MacMillan and Meckstroth[5] found that invasion of the potato sets by *Fusarium oxysporium* is a common cause of decay in Colorado. They found that this fungus did not invade the sets at 14° C or below, but that infection increased as the temperature was raised above this point. Ajrekar and Rana-dive[1] found, in India, that bacteria and *Sclerotium* sp. were associated with the decay of potatoes at temperatures above 20° C. Probably these facts explain the prevalence of seed piece decay in plantings during hot weather. The decay usually begins in that portion of the set which comes from the center of the tuber. In that region the cells are larger and are less densely filled with starch and other substances than are the cells near the surface. Appel[2] and other workers have noted that the cut surface of a tuber "heals" so that a new protective layer is formed, which excludes fungi as effectively as the original periderm of the tuber. Appel found that this layer appeared 48 to 60 hours after cutting, and was completed in two additional days. Shapovalov and Edson[18] found that the new periderm or protective layer generally developed much less toward the center of a tuber than near the periphery. This may be the reason that decay of the set usually begins in the part coming from the center of the tuber.

Priestley and Woffenden[13] studied the conditions under which the healing process takes place. They find that a warm moist atmos--phere with normal oxygen content is most favorable. They show that under these conditions, the cut surface is "blocked" in 24 to 48 hours by a deposit of fatty substances on the wounded surface. Two to five days later, a cork layer forms, as a result of rapid cell division, below the blocked surface. This results in a permanent protective layer.

With the idea that the decay of the sets in plantings during warm weather might be prevented by allowing a period for the development of the protective periderm before planting, experiments were conducted in 1925. Somewhat immature tubers were harvested July 9 and 11, samples were cut, sacked, and stored at different temperatures until July 28, when they were planted together with appropriate check lots that were cut at planting time. The results are given in table 9.

The lots cut at harvest time and stored at 1° C gave only a slightly higher percentage of stand and sprouted more slowly than the checks. Examination of the sets upon removal from storage indicated that periderm formation did not take place, to any marked extent, at 1° C. The other cut lots in storage at higher temperatures showed decay in direct proportion to the increasing temperature. Furthermore, the sprouting was much retarded in these lots, indicating that the dormant condition was prolonged, or that a secondary dormancy was

TABLE 9

EFFECT OF CUTTING IN ADVANCE UPON DECAY OF THE SETS AND RATE OF SPROUTING.
STORAGE PERIOD 16 TO 18 DAYS. SUMMER, 1925

	Coldframe planting			Field planting		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose						
Cut July 9—stored at 1°C	30	76 6	34 0	170	65.3	39 3
Cut July 9—stored at 7°C	30	30 0	.	170	48.8	50.9
Cut July 9—stored at 13°C . . .	30	26 7	. ...	175	38.3	45 5
Cut July 9—stored at 22°C .	30	10 0	.	170	31 2	50 0
Cut July 8, check	30	66 6	25 7	175	64 6	32 2
Idaho Rural						
Cut July 11—stored at 1°C	30	100 0	65.7	170	47 6	58.9
Cut July 11—stored at 7°C	30	93 2	64.1	170	47.0	68.3
Cut July 11—stored at 22°C	30	10 0	....	160	15.0	65 5
Cut July 28, check	30	90.0	55.7	150	49.3	57.6

induced, by cutting in advance of planting and storing at intermediate temperatures. The cuts set stored at 7°, 13° and 22° formed a periderm over the wounded surface, which interferred with gas exchange, hence retarded sprouting, yet did not protect the sets from decay when planted in the soil.

A similar experiment was conducted in 1927, using shorter storage periods for the cut sets, which were placed in shallow trays in the different storage chambers. The results are presented in table 10.

As in the previous experiment, the sets cut in advance of planting decayed to a greater extent than those cut at planting time. The retardation of sprouting in the suberized sets is also evident. The lot stored at 4°, which became only slightly suberized, and the lot stored at 22° in wet sawdust, showed the least decay and sprouted somewhat more rapidly than the check.

No means of preventing decay of cut sets under high temperature conditions has yet been found.   The suberization and healing of the cut surfaces prior to planting offers a promising line of attack for further investigation, however.   Meantime, practical advantage may be taken of the fact that cut sets from medium to small tubers are less subject to decay than sets from large tubers; that tubers fully matured before harvest have less decay than those harvested immature; and tubers stored at high temperatures (22°–30°) or under moist conditions at lower temperatures, decay less when planted than tubers which have been held in cold storage.   Those factors which make for more rapid sprouting also generally give a better stand.

TABLE 10

EFFECT OF SUBERIZATION OF CUT SETS UPON DECAY AND RATE OF SPROUTING.
WHITE ROSE VARIETY.   SUMMER OF 1927

	Coldframe planting			Field planting		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
Cut and stored for 6 days at						
4°C, dry.....	30	50 0	30.9	107	45 8	37.3
12°C, dry...........	30	40 0	40 5	114	38.6	46.5
22°C, dry..................	30	7.0	53 5	111	26 1	47.4
22°C, in wet sawdust	30	60 0	31.2	117	67 5	35.1
Room temperature, 15–35°C	30	0	....	100	20 0	59.8
Cut and stored for 13 days at						
4°C, dry...........	30	56.7	30.6	110	61.0	41 7
22°C, dry...........	30	7 0	69.0	110	39.0	47.6
Check—cut at planting	70	68.6	· 33.5	117	65 2	37 3

SUMMARY AND CONCLUSIONS

The more nearly mature potato tubers are when harvested, the shorter is the dormant period, as indicated by more rapid sprouting when they are cut and planted. ·

The rate at which mature tubers sprout increases rapidly with the length of storage before cutting and planting.   Tubers harvested immature emerge from dormancy more slowly.   The emergence from the dormant condition is a gradual, not a sudden change.

The temperature at which potatoes are stored influences the rate of emergence from the dormant condition, but previous claims as to the hastening of sprouting by cold storage are not substantiated.

Storage at 4° C may retard sprouting somewhat, compared to storage at 20° to 23°, when plantings are made in the early or middle portion of the dormant period. Storage at 28° to 30° has a marked accelerating effect upon subsequent sprouting, as compared to lower temperatures.

The humidity of the storage has little effect upon dormancy at very low temperature (4°), or at high temperature (30°). But at intermediate temperature (22°), tubers stored under moist conditions sprout much more rapidly after they are cut and planted.

The primordia of the vegetative sprouts develop during the later stages of tuber growth, as well as during the dormant period. At temperatures favorable for growth, the meristematic region is probably never entirely inactive. In the emergence from the dormant period, this activity increases greatly.

The normal duration of the dormant period for potato varieties differs, as well as their response to dormancy-breaking treatments.

One of the difficulties in growing a fall crop of potatoes in California is the prevalence of seed piece decay when the planting is made during hot weather. The amount of decay is increased and the dormancy of the sets is prolonged by cutting several days before planting and allowing the cut surface to become suberized.

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EFFECTS OF CHEMICAL TREATMENTS ON DORMANT POTATO TUBERS

J. T. ROSA[1]

FOREWORD

The treatment of dormant tubers with various substances to hasten sprouting has been extensively investigated, but as yet no method of treatment has been adopted by commercial growers. While it is believed that such matters as tuber maturity, storage temperature, and the normal length of dormant period for the variety are important factors, the use of chemical stimulants for seed treatment is also likely to be of practical value in many cases. The word "stimulant" is used to designate substances which are probably not used as nutrients, yet are capable of accelerating the plants' activities.

In this paper will be presented the results of a number of experiments, designed largely to determine effective methods for the stimulation of sprouting in dormant seed potatoes. Studies concerning the effect of such treatments upon the metabolism of the tuber are under way, but the discussion of this phase of the work will not be entered upon at this time.

PREVIOUS WORK WITH CHEMICAL TREATMENTS

McCallum[4] found that sprouting was hastened by exposing potatoes to the fumes of ethyl bromide, carbon tetrachloride, ammonia, and gasoline, at the rate of ½ to 1 cc. in a 5-liter chamber for 24 hours. He also obtained positive results with ethyl bromide and with ethylene chloride. Appleman[1] found that treatment with hydrogen peroxide gave positive results. Rosa[5] has reported hastened sprouting by soaking the cut sets in solutions of various salts before planting; 0.5 molar solutions of sodium and potassium nitrates for periods ranging from 10 minutes to one hour, 0.02 mol. potassium permanganate, .005 mol. ferric chloride, and .05 normal hydrochloric acid for

one hour, were all found to hasten sprouting, especially when the tubers were in the middle or later part of their dormant period. However, these materials were found to be somewhat toxic to the tissues of the tubers. While this had no bad effects when the sets were planted in fall, winter, or spring, when soil temperatures were low, there did result an increased amount of seed piece decay when the planting was in mid-summer, with high soil temperatures prevailing. Denny[2] tested 224 different chemicals on potatoes. He obtained striking results in hastened sprouting with ethylene chlorhydrin and ethylene dichloride applied in various way, and with sodium, potassium, and ammonium thiocyanate solutions. Even dormant tubers of the Irish Cobbler variety were caused to sprout in one month after planting by treatment with 2 or 3 per cent solutions of the thiocyanates. Denny also obtained favorable results with carbon bisulfide and some other substances.

Three varieties, White Rose, Idaho Rural, and Irish Cobbler, have been used in the writer's experiments. Tubers were grown at Davis in the regular spring crop, harvested just before the plants died, and placed in storage at 22° to 23° C. The storage period in most cases was three to four weeks. Plantings were made in sand in the coldframe, and in soil in the field. Counts were made every few days of the sprouts, as they emerged above the surface of the soil.

EXPERIMENTS WITH ETHYLENE

In 1925, two experiments were carried out, to determine the effect of ethylene gas on germination of potatoes. In the first test, three varieties were used. The tubers were harvested June 20, when they were approaching maturity. They were stored in a dry cellar at about 22° C. The samples to be treated for four weeks were placed in the gas chambers on June 24 and treatment was continued until July 23. The samples to be treated for two and three weeks were held in the cellar with the checks until the proper date, and then removed to the gas chamber. On July 26 (36 days after harvest) the tubers were cut and planted in the coldframe. The chambers were about 400 liters capacity, and were not absolutely airtight. Sufficient ethylene gas was applied each day to give a concentration of 1:400 in one chamber and 1:2200 in the other. The chambers were opened for a few minutes each day for ventilation. As the results with the two concentrations paralleled each other, they are averaged in table 1.

With these three varieties, 4 weeks' treatment with ethylene resulted in more rapid sprouting than was the case with the untreated checks. The difference was slight with the White Rose variety, which apparently had nearly reached the end of its dormant period (having been held at 22° for 36 days). The lots exposed to ethylene for 3 and 2 weeks gave in every case a slower rate of germination than the checks. The reason for this fact may be the known toxicity of ethylene to vegetative growth, as the sprouts may have started slightly on the 2- and 3-week lots before they were placed in ethylene. These lots were held in the cellar for 1 and 2 weeks, respectively, before

TABLE 1

EFFECT OF ETHYLENE UPON SPROUTING.   JULY–SEPTEMBER, 1925

Treatment	Number of sets	Per cent stand	Average number of days to emerge	Average number of stems per set
White Rose				
Ethylene, 4 weeks	60	95.0	28.3	1.51
Ethylene, 3 weeks	60	93.4	39.6	1.38
Ethylene, 2 weeks	60	95.0	39.0	1.28
Untreated	90	84.5	30.8	1.20
Idaho Rural				
Ethylene, 4 weeks	60	75.0	22.2	1.18
Ethylene, 3 weeks	60	76.6	38.9	1.15
Ethylene, 2 weeks	65	87.7	43.4	1.09
Untreated	90	74.7	30.7	1.12
Irish Cobblers				
Ethylene, 4 weeks	29	93.2	42.0	1.30
Ethylene, 3 weeks	30	93.2	70.5	1.14
Ethylene, 2 weeks	29	86.3	56.8	1.12
Untreated	60	73.4	50.0	1.23

being placed in the gas chamber. All of the ethylene-treated lots gave a higher percentage of stand than the checks, and a slight increase in the average number of stems arising from each set.

A second experiment with ethylene was performed in the fall of 1925, fully matured, spring crop tubers harvested July 30 being used. Lots of 30 to 40 sets each were planted in the greenhouse at intervals after harvesting. The untreated tubers were stored at 20° to 23° C, while those treated with ethylene were kept at the same temperature in a chamber with ethylene at 1:800 concentration applied daily. The results are given in table 2.

With the White Rose variety, the plantings made after 6 and 15 days show a marked hastening of the sprouting of tubers exposed to ethylene. The later plantings showed little difference, however, as the

tubers had evidently passed their dormant period. In this case, fully mature tubers of a variety having only a short dormant period were involved. With the Idaho Rural, the ethylene-treated lots sprouted more rapidly at all stages, though the gain over the untreated checks was greatest at the 15 and 28-day periods. In all cases, there was a tendency for the number of stems per set to increase as the period between harvest and planting was lengthened. This was most marked with the lots stored in ethylene gas. Rosa[5] has previously called

TABLE 2

EFFECT OF ETHYLENE UPON SPROUTING, WITH REFERENCE TO THE STAGE OF DORMANCY. FALL, 1925

	Untreated			Ethylene 1:800		
	Per cent stand	Average number of days to emerge	Average number of stems per set	Per cent stand	Average number of days to emerge	Average number of stems per set
White Rose Planted after						
6 days' storage.............	67	55 9	1 00	100	36.9	1.07
15 days' storage..........	77	38.2	1.14	97	33.2	1.00
28 days' storage..	100	20.0	1.04	100	20.5	1.03
39 days' storage..............	100	18 2	1 23	100	17 0	1 36
55 days' storage..	100	21 2	1.17	100	17.0	1.58
Idaho Rural						
6 days' storage	63	77 3	1 00	90	71.4	1.00
15 days' storage	45	60 8	1 00	83	.29.5	1.21
28 days' storage	100	38.4	1 03	100	27 1	1.14
39 days' storage	100	30.2	1.17	100	22.0	1.21
55 days' storage	100	29.7	1 23	100	16.5	1.61

attention to the increase in number of stems, in potatoes planted after the end of the dormant period, compared to those which are still somewhat dormant.

EXPERIMENTS WITH ETHYLENE CHLORHYDRIN ON LARGE TUBERS

This substance is obtained commercially as a 40 per cent solution of the gas in water. The gas volatilizes rather slowly when the solution is exposed to air. It was used in the three ways suggested by Denny. (1) Fumigating whole tubers in a closed chamber for 24 hours, using 1 cubic centimeter to each liter of space in the chamber. (2) Soaking cut sets in a one-half per cent solution for one hour. (3) Dipping the cut sets in a 3 per cent solution (or stronger) for a moment, then placing them in a closed container overnight.

The tubers used in the 1926 tests were harvested June 19, in a slightly immature condition. They were stored at 23° C until July 13, a period of 24 days, except for the four lots which were treated and planted 14 days after harvest. The tubers should have been still in the dormant condition at the end of this period. The results are given in table 3.

TABLE 3

EFFECT OF ETHYLENE CHLORHYDRIN UPON SPROUTING OF CUT SETS FROM LARGE MATURE TUBERS. SUMMER, 1926

	Coldframe plantings			Field plantings		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose, planted 14 days after harvest.						
Lot No.						
52. Untreated...................................	60	77.4	47 8	0	....	....
50. Gassed 24 hours	30	80.5	27.5	0	....	. .... .
49. Cut sets in 1% solution for 1 hour	30	66.6	37.5	0	....	....
51. Cut sets dipped in 3% solution	30	19.3	41 8	0	. . ....	,..........
White Rose, planted 24 days after harvest.						
57. Untreated....	60	74.9	34 3	241	50.6	38.5
55A. Gassed 24 hours at harvest time........	30	73.4	24.4	43	46.5	30.1
53. Gassed 24 hours 2 weeks after harvest	30	70.0	23.9	82	72.0	26.8
61. Gassed 24 hours at planting time	30	86.6	20.9	98	49.0	39.4
60A. Cut sets in ½% solution for 1 hour	30	86.6	25.6	76	53	31.8
60B. Same solution as in 60A, second lot	30	83 2	23 2	0	....	....
60C. Same solution as in 60A, third lot.....	30	66.6	21 6	0	. ...	....
63. Cut sets dipped in 3% solution........	30	30.0	19.6	69	36	34.8
91. Cut sets dipped in 4½% solution......	0	.. ..	....	94	24.5	39.5
Idaho Rural, planted 24 days after harvest.						
68. Untreated—large tubers, cut.........	161	76.8	34.6	399	61.6	51.1
55B. Gassed 24 hours at harvest,......	30	56.6	21.6	104	68.0	29.7

The White Rose tubers treated and planted two weeks after harvest (lots 49, 50, and 51) showed marked hastening of sprouting, especially in lot 50, which was treated by method 1. Those treated by method 3, in a 3 per cent solution, were much injured.

Lots 55A, 53, and 61, planted 24 days after harvest, were medium to large tubers, treated according to method 1 just after harvesting, 2 weeks after harvesting, and just before planting. Denny suggested that the toxic effects of the treatment upon the tubers may be lessened by treating some days in advance of cutting and planting. The results in coldframe and field plantings do not agree entirely. In the former, the treatment was most effective when given just before planting,

while in the field the highest percentage of stand and the most rapid sprouting occurred in the lot treated two weeks after harvest. It is apparent that the treatment at all three periods had considerable effect in hastening sprouting, with no evidence of toxic effects. It may be concluded that seed tubers can be safely treated at any time between harvest and planting. Figure 1 shows the marked difference
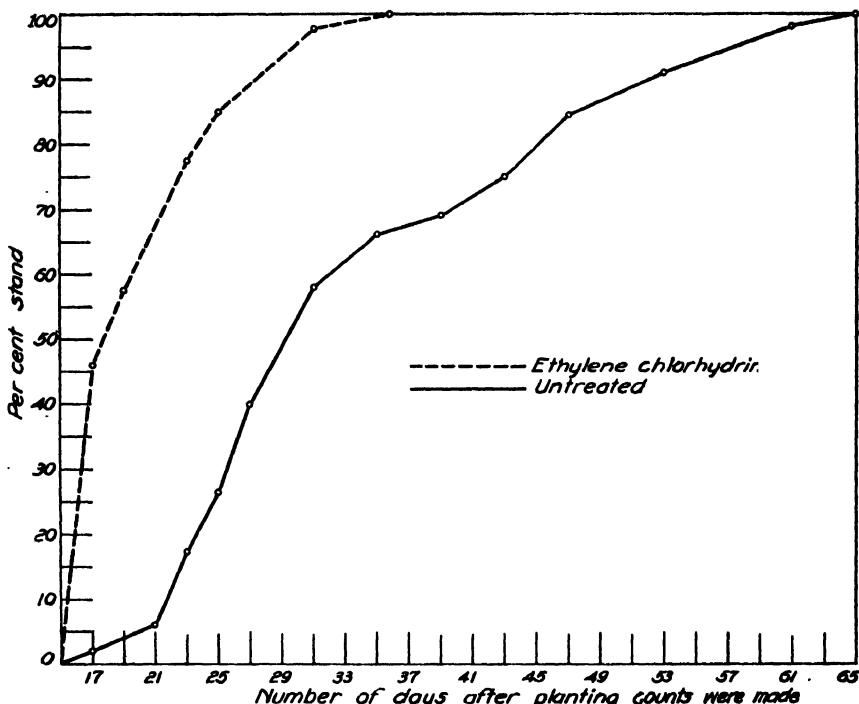
Fig. 1. The rate of sprout emergence of White Rose sets planted 24 days after harvest. Lot 57, untreated, and lot 61, treated with ethylene chlorhydrin just before planting, by method 1.

in rate of sprout emergence between untreated sets and those treated with ethylene chlorhydrin at planting time by method 1. While the treatments with ethylene chlorhydrin hasten sprouting, they do not, on the average, give a materially higher percentage of germination than no treatment, when cut sets from large tubers are considered. This is due, as in all the other experiments under high temperature conditions, to the decay of a large number of sets soon after planting.

With the Idaho Rural variety, large tubers treated with ethylene chlorhydrin at harvest time according to method 1 gave a very marked -acceleration of sprouting, there being an average gain of 13 days in the coldframe planting and 31.4 days in the field planting. However,

the percentage of the sets planted that produced plants was no larger than in the untreated lot.

Lots 60A, 60B, and 60C were treated according to method 2, the same solution being used to treat the three lots successively. The stimulation to sprouting was marked in all cases. Lots 63 and 91, treated according to method 3, again showed decided toxicity from this treatment, most of the seed pieces decaying.

On the whole, the results show that ethylene chlorhydrin is effective in hastening the sprouting of dormant potatoes, thus confirming

TABLE 4

EXPERIMENTS WITH ETHYLENE CHLORHYDRIN USED ACCORDING TO METHOD 1, ON LARGE TUBERS. SUMMER, 1927

	Coldframe planting			Field planting		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Averag number of days to emerge
White Rose						
Gassed 24 hours, 1 cc per liter....................	30	100.	21.7	107	73.8	25.4
Gassed 24 hours, 0.75 cc per liter..............	30	86.6	21.1	114	73.7	23.8
Gassed 24 hours, 0.5 cc per liter..............	30	76.6	22.4	120	44.2	27.5
Gassed 24 hours, 0.25 cc per liter.....	30	90.0	23.9	118	49.2	27.5
Checks—not treated.......................................	120	72.3	33.6	577	61.7	34.0
Idaho Rural						
Gassed 24 hours, 1 cc per liter....................	30	80.0	33.3	117	68.4	31.9
Gassed 24 hours, 0.75 cc per liter..............	30	83.3	29.4	107	59.8	30.9
Gassed 24 hours, 0.5 cc per liter..............	30	86.6	33.2	80	27.5	33.3
Gassed 24 hours, 0.25 cc per liter..............	30	70.0	32.0	86	41.0	38.9
Checks—not treated.......................................	90	57.7	35.6	554	56.6	37.9

Denny's work. However, treatment with this material does not prevent decay of the seed pieces under high temperature conditions, and in treatment by method 3, even increases it. This factor would probably not be so serious in cool localities or in winter and spring plantings. Of the various methods of using ethylene chlorhydrin, method 1 seems most likely to be suitable for commercial use.

Further experiments with ethylene chlorhydrin were conducted in 1927, to determine the optimum concentration for treatment by method 1. A tight chamber, $4 \times 4 \times 5$ feet in size, was used. The tubers were placed in shallow boxes stacked one above the other with strips between. An electric fan blowing directly upon a pan containing the ethylene chlorhydrin hastened its volatization. The treatments were made at 20° to 24° C. Denny[3] states that the dosage can be

reduced to .35 cc. per liter of space, when the treatment is performed in such a room, with a fan. The results obtained on the large tubers treated by this method are given in table 4. The treatments were made 10 or 11 days after harvesting upon tubers stored in a dry cellar at 22° C. The tubers were cut and planted 10 days later.

The White Rose responds somewhat more markedly to treatment with ethylene chlorhydrin than does the Idaho Rural, but both varieties show a marked stimulation of sprouting from treatment with this material. The sprouting is most rapid with 1 cc. and with 0.75 cc. per liter of space, the latter concentration being slightly more effective than the former. The lower concentrations, while exerting considerable effect, are markedly less stimulating under the conditions of these experiments. There was no evidence of toxic effects at any concentration, the percentage of stand being in most cases better than that of the checks.

Experiments with Ethylene Chlorhydrin upon Small Tubers

Because so many cut sets decay when planted in the field during summer, and since uncut tubers are not so subject to decay, it was thought that it might be advisable, from the practical point of view, to plant small tubers whole. Earlier experiments, however, had shown that sets of this kind sprouted very slowly. Experiments were made in the summers of 1926 and 1927 to determine the effect of various chemical treatments on such tubers. The results with ethylene chlorhydrin are given in table 5. Tubers, harvested mature, about 1.5 ounces in average weight, were treated according to method 1 at harvest time and just before planting, respectively.

Treatment with ethylene chlorhydrin in most cases increased the per cent stand, as determined by the last count at the end of the growing season, but the speed of sprout emergence was increased only slightly. In the latter respect, however, the tests of 1927 with both White Rose and Idaho Rural show distinctly more marked effects from treatment immediately after harvest than from treatment in the middle of the storage period or just before planting. The lesser degree of suberization of the periderm at harvest time than after storage for a period, may permit the ethylene chlorhydrin to enter the tissues of the tuber more effectively. The fact remains, however, that small tubers planted whole do not respond vigorously to the same treatment that proved very effective on large tubers of the same varieties and

degree of maturity, but which were cut before planting.   Similar results were obtained with ethylene dichloride and ethyl bromide, and are shown graphically in figure 2.   Cutting is itself a mild dormancy-breaking treatment, as shown by Appleman.[1]   Schlumberger[6] considers wound-irritation, as induced by cutting the tuber or by injury in various other ways, to be of great importance in stimulation effects. Such effects are lacking when the tubers are planted whole.

TABLE 5

EXPERIMENTS WITH ETHYLENE CHLORHYDRIN UPON SPROUTING OF SMALL TUBERS PLANTED WHOLE, 21 DAYS AFTER HARVEST

	Coldframe planting			Field planting		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose, 1926						
Lot 56A. Gassed at harvest	30	53.3	52 8	107	74 0	72 7
Lot 62. Gassed at planting	30	60 0	60 5	78	74 0	74 7
Lot 58A. Check—not treated	30	13.3	64 7	85	42 0	81 4
White Rose, 1927						
Lot 67. Gassed at harvest	0			102	90 0	43 4
Lot 71. Gassed after 10 days	0			115	90 0	49 6
Lot 74. Gassed at planting	0			115	86 0	50.6
Lot 38. Check—untreated	0			120	85 0	61 7
Idaho Rural, 1926						
Lot 56B. Gassed at harvest	30	56 6	52.4	98	85 0	70 9
Lot 56B. Check—untreated	30	83.2	56.6	109	82 0	81 1
Idaho Rural, 1927						
Lot 68. Gassed at harvest	0			117	71 8	52 8
Lot 72. Gassed after 10 days	0			120	83 3	61.5
Lot 75. Gassed at planting	0			120	74 2	65 0
Lot 39. Check—untreated	0			120	76.7	65.5

EXPERIMENTS WITH SODIUM THIOCYANATE

Denny[2] reported favorable results from soaking cut sets for one hour in 3 per cent solutions of sodium thiocyanate, when the tubers were deeply dormant, and in a 2 per cent solution for one hour or in a 1 per cent for two hours, as they approached the end of the dormant period.   Experiments were conducted in the summer of 1926 to determine the suitability of sodium thiocyanate for treatment of potatoes under California conditions.   Ammonium thiocyanate was used in 1927.   Large tubers of the White Rose variety, harvested quite immature, and smaller but mature tubers of the Idaho Rural and

Irish Cobbler were used. The difference in maturity and in tuber size may explain the less injurious effects secured with the last two varieties. It has been the general observation in all of these experiments that seed piece decay is more prevalent in·sets cut from large tubers, especially when they are harvested immature. Table 6 gives the results.

TABLE 6

EFFECT OF SODIUM THIOCYANATE AND AMMONIUM THIOCYANATE UPON SPROUTING

	Coldframe planting			Field planting		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
White Rose, 3 weeks after harvest.						
Lot 57. Untreated...............................	90	67.8	30.2	98	46 7	41.4
Lot 37. 2 per cent solution of NaCNS for						
½ hour......................................	30	70.0	35.4	89	50.6	35.3
Lot 38. 2 per cent solution of NaCNS for						
1 hour	30	66.6	33.0	105	63.8	34.8
Lot 39. Same, second lot..........	30	32.0	....	0	....	....
Lot 40. Same, third lot..............	30	16.7	....	0	....	....
Lot 41. Same, fourth lot	30	3.3	....	0	....	....
Lot 42. 3 per cent solution of NaCNS for						
½ hour...............................	30	26.7	28.7	106	46.2	36.8
Idaho Rural, 2 weeks after harvest.						
Lot 48. Untreated..........	30	90.6	45.8	0	....	....
Lot 47. 2 per cent solution of NaCNS for						
1 hour....................................	30	74.2	43.6	0	....	....
Idaho Rural, 3 weeks after harvest.						
Lot 67. Untreated......................................	161	76.8	34.6	399	61.6	51.1
Lot 34. 2 per cent solution of NaCNS for						
1 hour....................................	30	76.6	28 5	116	·77.6	32.3
Irish Cobbler, 24 days after harvest (1927).						
Lot 102. 2 per cent solution of NH₄CNS						
for 1 hour	35	31.6	33.5	120	21.6	38.0
Lot 103. 2 per cent solution of NH₄CNS						
for ½ hour............................	35	48.6	41.8	120	37.5	44.1
Lot 104. 1 per cent solution of NH₄CNS						
for 1 hour...............................	34	64 7	45.4	0	....	....
Checks—untreated.......	60	63.3	37.2	186	78.6	36.2

Soaking the White Rose sets in 2 per cent sodium thiocyanate for one-half hour and for one hour had little effect upon the percentage of stand or upon the time required for sprouting. The Idaho Rural variety treated in a 2 per cent solution for one hour, two weeks after harvest, showed little effect, but this treatment a week later gave marked hastening of sprouting without toxic effects. Treatment in 3 per cent solution for only one-half hour resulted in very severe injury to the sets. With the Irish Cobbler, treatment in 2 per cent

solutions of ammonium thiocyanate was very toxic, the amount of seed piece decay being much increased. The 1 per cent solution was not toxic, but had no effect upon the rate of sprouting. In other tests, in the greenhouse during the winter, the 3 per cent solution gave marked stimulation without toxic effects. With this material, as with others previously tested in California, the danger of toxic effects is greatly increased in hot weather. The thiocyanates, like some other substances, may be used safely in the cooler parts of the season, but cause much decay of the sets, with resultant poor stands, under high temperature conditions.

Lots 38, 39, and 41, which were treated successively in the same solution, show a decreasing stand, indicating increased toxicity of the thiocyanate solution. This may be due to differential absorption of ions by the potato tissue, thus altering the composition of the solution. Apparently sodium thiocyanate solution cannot be used repeatedly in treating potatoes.

OTHER CHEMICAL TREATMENTS

Several chemicals besides those previously discussed have been tested from time to time, to determine their effect upon sprouting. Since time and concentration factors are involved, as well as the stage of dormancy of the tuber and the temperature at which the plantings are made, this is a slow and laborious process. In the winter of 1924–25, tests were made in the greenhouse of immature White Rose tubers of the fall crop harvested November 15. Table 7 gives the results of some treatments which had positive results.

The immature tubers treated only 2 days after harvesting on November 17 showed marked stimulation by ethyl bromide. Whole tubers (which were cut before planting) of the stage of maturity used in these tests cannot be safely treated for more than 15 minutes at the concentration employed. Cut sets did not respond as well as those treated whole and then cut for planting. The time and concentration factors should both be much lower where cut sets are to be treated.

Potassium dichromate was mildly stimulating, but was toxic at the higher concentrations, the tissue around the eyes being chiefly affected, and not the pith parenchyma (inner medulla) as is the case with most toxic substances. Potassium ferro- and ferricyanide, tested at this time in concentrations ranging from .05 mol. to .25 mol. solutions for one hour, showed both forms of injury, and no stimulating effects.

In the tests planted January 10, the tubers were nearing the end of the dormant period. Whole tubers were treated with the various materials, then cut and planted at once. Ethyl acetate and ether showed considerable stimulation, while carbon tetrachloride and gasoline had less effect. The last two substances, tested at higher concentrations, proved toxic. Chloroform was decidedly toxic, and

TABLE 7

MISCELLANEOUS CHEMICAL TREATMENTS IN WINTER OF 1924–25

Date and treatment	Number of sets	Per cent stand	Average number of days to emerge	Injury to sets
ov. 17. Ethyl bromide on whole tubers, cut for planting.				
4 cc per liter for 15 minutes..	19	84	31.4	slight
4 cc per liter for 30 minutes..	20	60	31 4	severe
4 cc per liter for 60 minutes..	17	35	....	very severe
Untreated..	35	90	92.0	....
Ethyl bromide on cut sets.				
2 cc per liter for 2½ minutes	20	100	49.7	none
2 cc per liter for 5 minutes......................	19	100	43.8	none
2 cc per liter for 7½ minutes......	19	95	54.2	very slight
2 cc per liter for 10 minutes......	17	94	50 5	slight
2 cc per liter for 20 minutes.........	19	79	44.3	severe
Dec. 10. Potassium dichromate—cut sets.				
Soaked 1 hour in .01 mol. solution..............	18	67	59.5	eyes injured
Soaked 1 hour in .005 mol. solution................................	17	88	66.9	none
Soaked 1 hour in .001 mol. solution	17	100	53 4	none
Untreated.....	36	100	- 70.0	....
Jan. 10. Whole tubers, cut for planting.				
Ether, 2 cc per liter for 1 hour..........................	21	100	22.9	none
Ethyl acetate, 2 cc per litre for 1 hour	20	100	21.4	none
Chloroform, 2 cc per liter for 1 hour	17	47	....	severe
Carbon tetrachloride, 2 cc per liter for 1 hour.............	18	100	25.1	none
Gasoline, 4 cc per liter for 1 hour..................................	17	100	25.2	none
Untreated............................	21	100	29.4	....

bromine and chlorine gases, which were tested at one-half and at one hour exposures, were very toxic.

In the summer of 1925, tests were made on tubers harvested nearly mature, the results of which are given in table 8. Whole tubers were exposed to ethyl bromide for 15 minutes at the same concentration as before. This treatment, upon well suberized tubers after one month's storage gave little stimulation; the results were not so striking as in former tests, with immature tubers. Small tubers treated 10 days after harvest for 15, 30, and 60 minutes, and planted without cutting, showed very little stimulation with the time and concentration used

TABLE 8

EFFECT OF MISCELLANEOUS CHEMICALS UPON SPROUTING.   SUMMER, 1925

(Coldframe planting—30 sets in each lot)

	White Rose		Idaho Rural	
	Per cent stand	Average number of days to emerge	Per cent stand	Average number of days to emerge
Large tubers 1 month after harvest.				
Ethyl bromide 15 minutes...	97	27.8	97	44.0
Untreated..	84.5	30.8	72	37.3
Small tubers 10 days after harvest.				
Ethyl bromide for 15 minutes..................................	0	....	97	76.6
Ethyl bromide for 30 minutes..................................	0	....	80	71.0
Ethyl bromide for 60 minutes..................................	0	....	82	70.9
Untreated..	0	....	93	80.0
Tubers 17 days after harvest—cut sets.				
Sodium nitrate, .5 mol. solution, soaked 10 minutes......	90	21.0	77	31.2
Sodium nitrate, .5 mol. solution, soaked 20 minutes.....	87	22.8	63	27.9
Sodium nitrate, .5 mol. solution, soaked 30 minutes.....	63	23.5	27	27.0
Untreated..	84	33.5	90	55.7
Potassium permanganate, .02 mol. solution, soaked 15 minutes	77	33.1	77	36.5
Potassium permanganate, .02 mol. solution, soaked 30 minutes	70	29.0	72	37.0

here. Subsequent experiments with ethyl bromide, however, using 0.25 cc. to 0.5 cc. per liter for 24 hours upon whole tubers, gave a marked stimulation to sprouting, without toxic effects.

Treatment of cut sets 17 days after harvest in 0.5 mol. solution of sodium nitrate gave a marked stimulation to sprouting, especially in Idaho Rural variety. Under the high temperatures prevailing when these tests were made, even a 20 minute soaking in nitrate solution caused some injury, and a 30 minute one was very toxic. Potassium

TABLE 9

EFFECT OF MISCELLANEOUS CHEMICALS UPON SPROUTING.   SUMMER, 1926

	Coldframe plantings			Field plantings		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cent stand	Average number of days to emerge
Untreated..	161	76.8	34.6	399	61.6	51.1
Ethylene dichloride, whole tubers...............................	45	68.9	30.2	106	73.0	44.3
Ethylene dichloride, cut sets......................................	30	96.7	33.2	96	51.0	47.6
Sodium nitrate, 0.5 mol. for 20 minutes........................	77	78.3	29.0	203	69.0	42.1
Ethylene 1:2000, gassed for 3 weeks............................	30	83.2	27.5	99	66.0	45.3
Propylene 1:2000, gassed for 3 weeks............................	60	83.3	27.5	182	44.0	44.8

permanganate at 0.02 mol. concentration up to 30 minutes soaking proved to be mildly stimulating and non-toxic, but longer treatment or higher concentrations of this substance proved injurious in other tests. The permanganate is reduced rapidly by contact with cut potato sets.

In the summer of 1926 ethylene dichloride, sodium nitrate, ethylene and propylene were tested upon Idaho Rural tubers harvested nearly mature and stored for 24 days. The results are given in table 9.

Ethylene dichloride was one of the substances which gave especially good results in Denny's experiments. In the tests here, it was used at the rate of 0.114 cc. to a liter of space, for 24 hours on whole tubers which were afterwards cut for planting, and half this dosage for 15 hours on cut sets. It proved to be moderately stimulating to sprouting but did not prevent the usual large percentage of seed piece decay. The same can be said for the other substances tested.

FURTHER EXPERIMENTS WITH ETHYLENE DICHLORIDE

Further experiments were made in the summer of 1927 with ethylene dichloride upon Idaho Rural and Irish Cobbler. These two varieties had been previously found not to respond as vigorously to ethylene chlorhydrin as does the White Rose. The tubers used were harvested nearly mature and stored in a dry room at 22° for three weeks before planting. They were, accordingly, well suberized at the time the treatments were made, which was two weeks after harvest and one week before planting. The treatment was by the vapor method, the proper amount of liquid ethylene dichloride being placed with the dry, whole tubers in a chamber which was kept closed for 24 hours. The tubers were of two classes, large tubers that were subsequently cut for planting, and tubers of one to one and one-half ounce weight, which were planted whole. The results are given in table 10.

Considering first the effect of ethylene dichloride upon large tubers, it appears that there are no toxic effects with either of the concentrations used, since the percentage of stand is in all cases higher than in the untreated lots. It is also evident that this material exerts a marked stimulating effect upon both varieties tested. The evidence is not conclusive as to which concentration is most effective, but the higher concentration appears to be somewhat more so.

In the tests with small tubers planted whole, some increased stand resulted in every case, from treatment with ethylene dichloride, due

TABLE 10

EFFECT OF ETHYLENE DICHLORIDE UPON SPROUTING OF CUT SETS FROM LARGE
TUBERS, AND OF SMALL WHOLE TUBERS. 1927

	Coldframe planting			Field planting		
	Number of sets	Per cent stand	Average number of days to emerge	Number of sets	Per cen stand	Average number of days to emerge
Idaho Rural, large tubers.						
Lot 98. 0.2 cc per liter............	30	83.3	43.8	97	75.3	42.2
Lot 99. 0.4 cc per liter............	30	96 7	25.5	111	68.5	31.2
Checks—untreated.................	90	57.7	35 6	554	56.5	37.9
Irish Cobbler, large tubers.						
Lot 100. 0.2 cc per liter.........	30	100.0	30.8	115	83.5	24.1
Lot 101. 0.4 cc per liter...	30	96.7	29.1	115	81.7	26.8
Checks—untreated...............	60	80.0	37.2	136	78.6	36.2
Idaho Rural, small tubers.						
Lot 87. 0.2 cc per liter.......... .	0	....	....	98	75.5 ·	57.1
Lot 88. 0.4 cc per liter......... .	0	....	....	103	90.2	53.3
Check, untreated..................	0	.. ..	....	120	76.7	65.5
Irish Cobblers, small tubers.						
Lot 93. 0.2 cc per liter..........	0	....	....	100	88.0	48.3
Lot 94. 0.4 cc per liter..........	0	....	....	120	83.4	50.0
Check—untreated..................	0	....	....	105	71.4	65.0
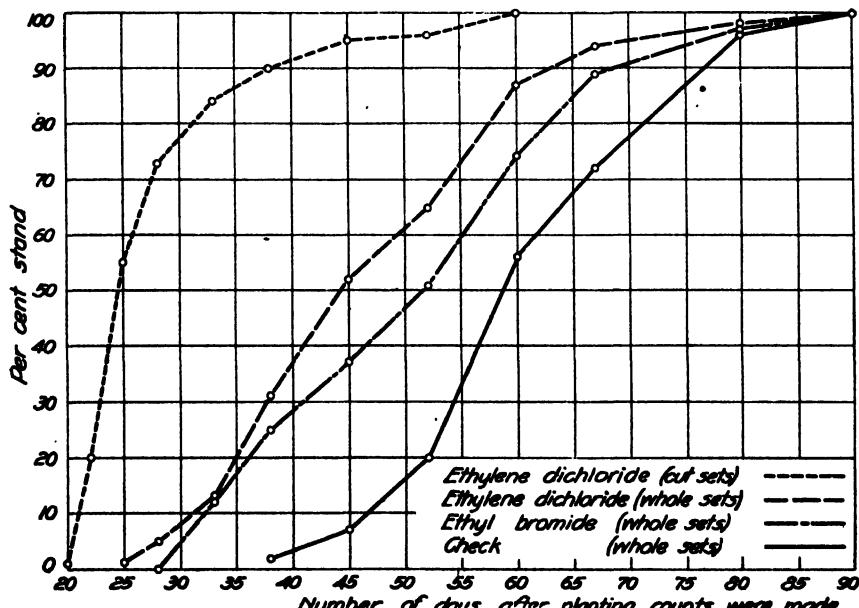
Fig. 2. The rate of sprout emergence of small tubers planted whole, untreated
and after treatment with ethylene dichloride and ethyl bromide, compared to
cut sets from large tubers treated with ethylene dichloride. Irish Cobbler
variety.

to the fact that more plants appeared above ground before the end of the growing season. Furthermore, there is some increase in the rate of sprouting, though here as in other experiments with tubers planted whole, the stimulatory effect is less than in tubers cut before planting. Whereas the treatment of large tubers reduced the average time required for emergence by about 30 per cent, with small whole sets this period was reduced only 15 per cent in Idaho Rural, and 26 per cent with Irish Cobbler. Figure 2 shows that whole tubers planted in the field, after treatment with ethylene dichloride and ethyl bromide, sprout more rapidly than untreated whole tubers, yet they lag considerably behind cut sets of the same variety.

SUMMARY

Ethylene, in concentrations of from 1 : 400 to 1 : 2200 of air, exerted a mild effect upon the hastening of sprouting of dormant, nearly matured, moderately suberized tubers placed in the gas chamber at harvest time and held there for four weeks.

Ethylene hastened the sprouting of fully matured White Rose tubers after treatment for only six days, while the Idaho Rural variety showed maximum stimulation after treatment for fifteen days.

Ethylene chlorhydrin proved to be a very effective material for treating the White Rose and Idaho Rural varieties, especially the former, at 14- and 21-day periods after harvest. Treatment with this material by the gas method on whole tubers and by soaking cut sets in a ½ per cent solution for one hour, showed marked stimulation to sprouting without toxic effects. A third method, dipping cut sets in 3 per cent or stronger solutions for a moment, then storing over night in a closed container, resulted in a great increase in the decay of the sets after planting.

Treatment of large tubers with ethylene chlorhydrin by method 1, at different stages during the storage period did not give consistently different results. With small tubers planted whole, however, the most marked stimulation effect was observed when the treatment was given at harvest time, when the tubers were only moderately suberized.

Tests to determine the optimum concentration of ethylene chlorhydrin to use according to method 1, indicate that for large tubers that are well suberized, the most effective concentration is 0.75 cc. per liter of space. This is for treatment in a room at 20°–25° C, with a fan to hasten the volatilization of the gas, as well as to insure uniform distribution.

Sodium thiocyanate and ammonium thiocyanate, especially the latter, were found to be toxic to cut sets soaked in 2 and 3 per cent solutions, when planted during hot weather. One per cent solution was non-toxic and had little or no stimulating effect.

Ethyl bromide was found to be an effective material for hastening sprouting, but the proper concentration for treatment varies widely, according to the maturity and degree of suberization of the tuber skin. This substance was not found to be satisfactory for the treatment of cut sets.

Sodium nitrate was moderately effective as a stimulant, when cut sets were soaked in an 0.5 mol. solution. However, this material is likely to cause excessive decay of the sets when the planting is made in hot weather.

Ethylene dichloride was very effective, especially on the Irish Cobbler variety, when used either as a gas on whole tubers or in solution for cut sets. The former method seems to be the most practical one. The optimum concentration probably lies between 0.2 and 0.4 cc. per liter of space, for a 24-hour exposure on large tubers.

Experiments with ethylene chlorhydrin, ethylene dichloride, and ethyl bromide upon small mature tubers that are planted whole, indicate that treatment of this class of tubers is much less effective than similar treatments upon large tubers that are cut before planting. It is not known whether this difference is due to a naturally longer and more profound dormancy in small tubers, or to the additive stimulation effect of cutting upon tubers previously treated with stimulants. From the practical viewpoint, however, of elimination of seed piece decay and of stimulating prompt sprouting of potatoes planted during midsummer, there is considerable promise for combinations of storage methods known to hasten sprouting with the use of chemical stimulants upon small tubers.

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SHAPE OF THE WATER TABLE IN TILE DRAINED LAND

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In 1915, while investigating drainage conditions at Kearney Park, California, the author was attracted by what appeared to be a discrepancy between the shape of the water table profile between lines of tile as found in the field and those usually shown in textbooks and other published papers on drainage. At the time, the shape of this profile was attributed to the heavy flooding which was being done on the drained tract.
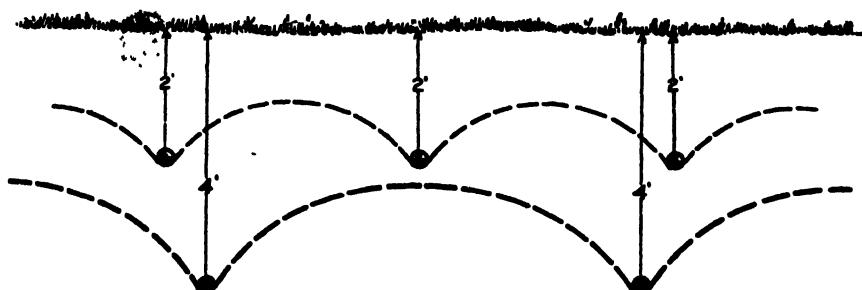
Fig. 1. A diagram similar to those commonly used to illustrate the shape of the water table between tile lines but which shape is not in accordance with the findings herein described.

A year or two later similar characteristics were observed on tile drained tidal marsh lands in Marin County, and this was attributed to the very heavy-textured soil of this area. It is now believed, however, that these observed shapes were not due to unusual or particular conditions, but that they represent the normal conditions of the water table on drained land.

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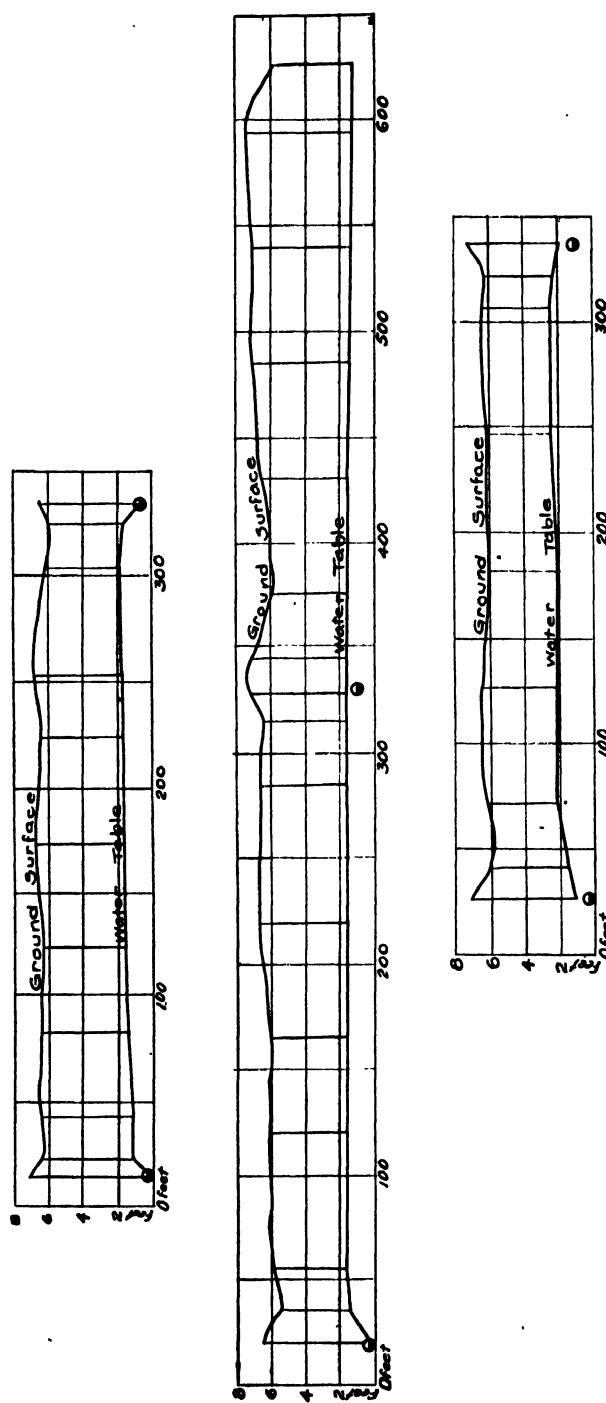
Fig. 2. Water table profiles in sandy loam soil at Kearney Park, California. Points at which measurements were taken are indicated by vertical lines from the surface to the water table.

Figure 1, which is taken from one of the author's own papers, is illustrative of the general type of information on the shape of the water table to be found in many publications. Although in this, as in most of the publications, the illustration was used for another purpose than to show the shape of the curve, it leaves what the writer now believes to be an erroneous impression in the mind of the reader.

SOURCES OF DATA

The data obtained at Kearney Park, California, in 1915, has been used to construct the profile shown in figure 2. In this drainage system the tile are in parallel lines 315 feet apart and about 6 feet deep. All of the lateral drains are 6 inches in diameter. The soil on the Kearney Park Tract is a fine sandy loam containing noncontinuous layers of hardpan, which, however, does not appear to interfere with the downward movement of water.

In Marin County, the area from which data were obtained is a heavy-textured tidal marsh from which tidal overflow is prevented by dikes. The tile lines, consisting of 4-inch tile, were 190 feet apart and between 3 and 4 feet in depth. Figure 3 shows the water table profiles from this tract.

In order to obtain more detailed and complete information regarding the shape of the water table than was available from the work at Kearney Park and in Marin County the investigations described more in detail were conducted in the Newhope Drainage District of Orange County during the summer of 1926.

This District contains about 4,000 acres of tile drained irrigated land and is situated on the west side of the Santa Ana River, directly west of the city of Santa Ana.

The soil of this area is Hanford sand and fine sandy loam. This is a recent alluvial deposit which is deep and readily permeable to roots and water.

The drainage system consists of lines of tile located in roughly parallel, north and south lines, about one-quarter mile apart. The tile used in this system vary in size from 30 inches in diameter at the lower end of the main line to 8 inches in diameter at the upper ends of laterals. The average depth of drain is between 8 and 9 feet. The water table has been quite generally lowered over the district, as indicated by measurements taken both before and after the drainage system was installed. In many places this has amounted to 3 feet
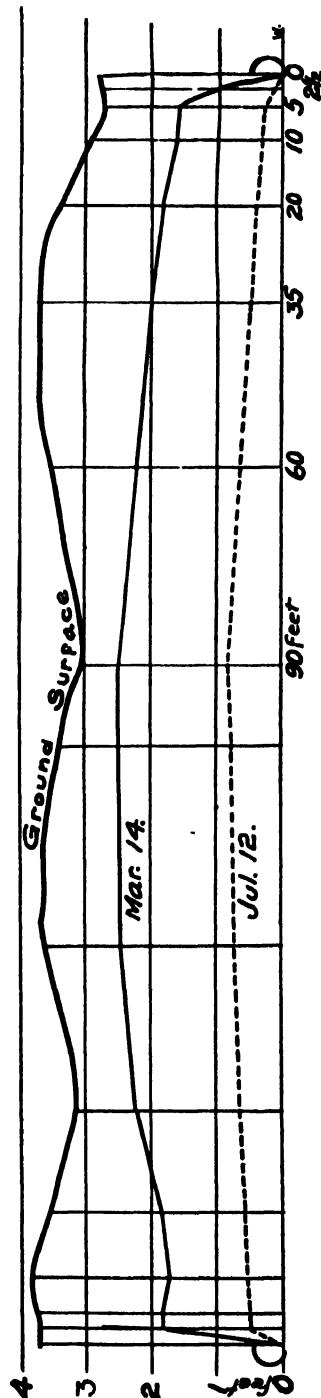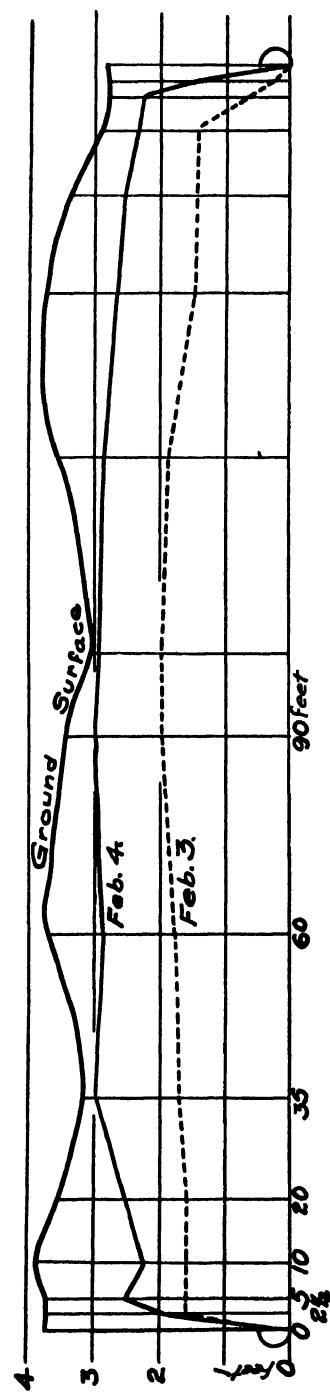
Fig. 3. Water tables profiles on a reclaimed tidal marsh in Marin County, California. Vertical lines indicate points at which measurements were taken. Profiles are from the same points but on different dates.

or more.   Figure 4 is a map of the district showing the location of the drains with respect to the boundaries of the district.

This district appeared to have almost ideal conditions for the study of water table profile shapes because the soil is fairly uniform in texture, depth and general characteristics.   The drains run princi-pally all in one direction and far enough apart to provide for full
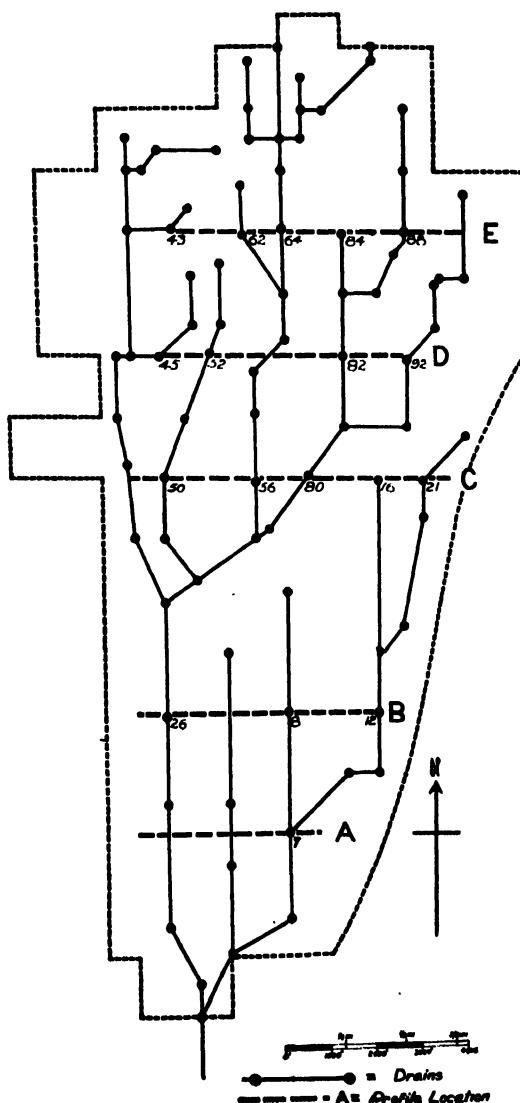
Fig. 4.   Map of New Hope Drainage District showing location of drains and profiles.   Only those manholes at which measurements were taken are shown numbered in the map.
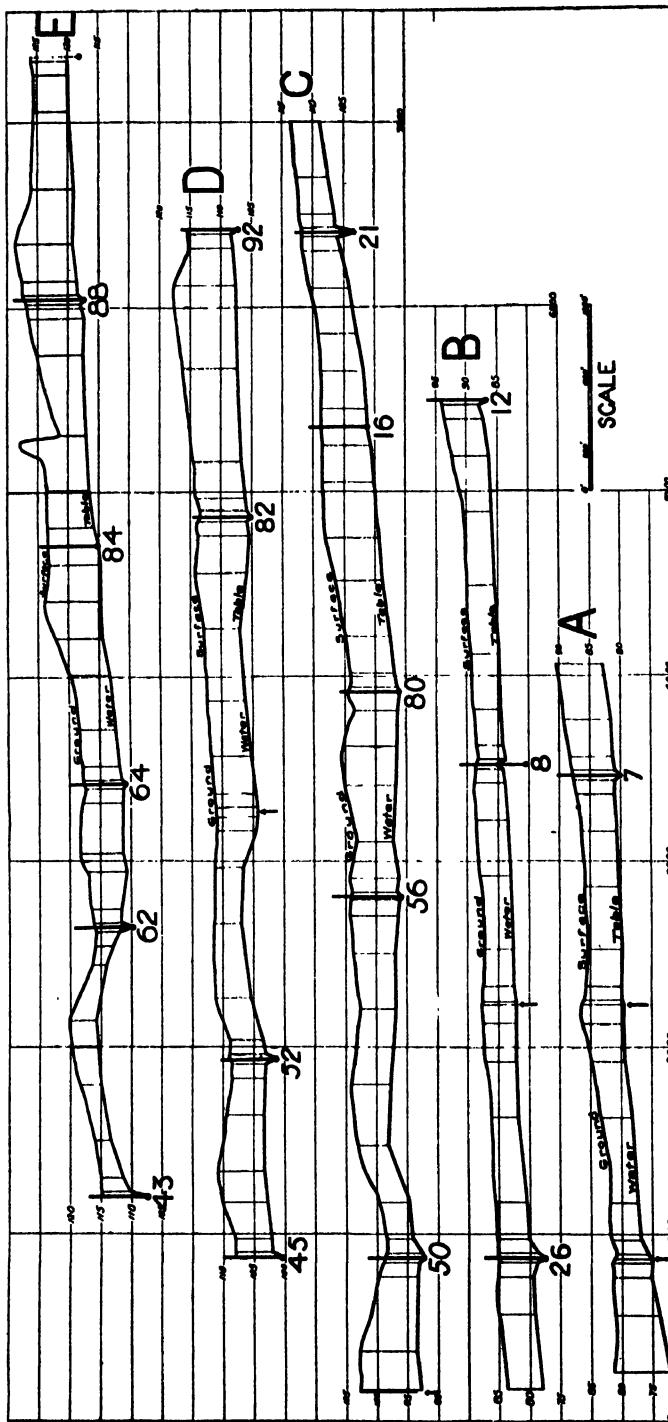
Fig. 5.   Profiles of water table in Newhope Drainage District.   The profile designations and manhole numbers correspond with those taken in figure 4.   Light vertical lines indicate points where measurements were taken.

development of water table profiles. The drains are also deeper than usual and the tract is satisfactorily drained.

The field work consisted of locating five profile lines across the district as shown in figure 4, and of boring down and determining the depth to water at frequent intervals along these lines. The elevation of the ground and the water table at each point and the relation between one point and another was determined by a line of levels. The elevations used, however, are from an assumed datum. These profile lines were located along the roads in order to make use of the greatest number of manholes, which are more or less regularly located along the tile lines, as points where the elevation of the water as it stood in the tile could be measured. There are only three instances in which there was no manhole at the point where the profile line crossed the tile line.

Profile A, the most southerly, is through the center of section 21, T 5 S., R 10 W., S.B.B.& M. It is 3,790 feet long, crosses three tile lines and contains 25 points of observation.   .

Profile B, one-half mile north of A, is along Smeltzer Avenue. It is 5,310 feet long, crosses four tile lines and contains 31 points of observation.

Profile C, one mile north of B, is along First Street. It is 6,840 feet long, crosses six tile lines and contains 42 points of observation.

Profile D, one-half mile north of C, is along Hazzard Avenue. It is 5,540 feet long, crosses five tile lines and contains 34 points of observation.

Profile E, the most northerly, and one-half mile north of D, is along Seventeenth Street. It is 6,160 feet long, crosses six tile lines and contains 40 points of observation.

There is some variation in the spacing of the test points on the profile lines due principally to a variation in distance between tile lines, but in the main an observation was made directly over a tile line, usually through a manhole and at 25 feet, 100 feet, 300 feet and 600 feet on either side of the tile lines. These points, as well as the distance from the ground surface to the water, are shown in figure 5.

ANALYSIS OF DATA

The significant feature of the profiles shown in figure 2 is that, except within 10 or 15 feet of the tile line, the water table is practically horizontal. There is a slight rise in the water table at mid points between tile lines, but in no case is this rise more than one foot and in most instances only a few inches. The fact that the general water table stands a foot or more above the tile has no particular significance so far as the shape of the water table curve is concerned.

In figure 3 it will be noted that beyond about five feet from the tile line, the water table is practically horizontal. The shape of the water table surface is not materially changed by the depth to water as the four profiles are, for all practical purposes, parallel.

The data secured from the Newhope Drainage District is presented in figure 5. Here, as in the work previously referred to, the water table assumes a nearly horizontal position between tile lines with a very sharp and marked depression immediately over the drain. As has been mentioned, many writers have implied by drawings and otherwise that the surface of the water table takes a much more rounded curve between tile lines than has been found to be the case in these studies.

It can be seen from the profile that with only about three exceptions the water table at any point more than 100 feet from the tile line is very nearly at the same elevation that it is 600 feet or midway between lines. It should be remembered in considering this point that the water table has been lowered about three feet over the entire district as the result of the drainage system.

Unless there was a manhole within 50 feet of the point where the profile line crossed the tile line, the elevation of the water table in the tile was not obtained, but in three such cases borings were made directly over the line.

On profile A (fig. 5) the only place where the water in the tile was measured, was at manhole 7. On profile B the water stood very high in manhole 8 due to a stoppage in the tile below this point; in fact, the water stood considerably higher in the manhole than it did in the immediately surrounding soil. For a considerable distance along the north side of profile B, the land was planted to peppers which receive heavy weekly irrigations. This fact, together with that of the obstructed tile, undoubtedly accounts for the water table being so near the surface along this line. On profile C the west

slope and top of the sandy ridge between manholes 50 and 56 was irrigated the afternoon and evening before these measurements were taken. This probably accounts for the high water table at the 4th and 5th observation east of manhole 50. Manhole 16 is at the upper end of a drain. This is also true of manhole 84 on profile E. The high water table between manholes 52 and the next tile line to the east on profile D may have been caused by a recent irrigation about midway between these two lines of tile, and the extremely high water table between manhole 43 and 62 on profile E is unquestionably due to the very heavy irrigation of a pepper field on the high ridge just north of this profile line. It can be seen that this has affected the water table even beyond the tile line on which manhole 62 is located.

It does not appear that the shape of the water table midway between tile lines is materially affected by the fact that the table is considerably above the tile line as on profile B or it is on the same level as at manholes 16 and 84. It is not definitely known that the elevation of the water in a flowing tile line actually represents the elevation of the water table in the soil even immediately adjacent to the tile. Evidence tending to show that this is not the case can be found at four points where borings were made directly over the tile, as at manhole 62 and one point on profile B and at two points on profile A. At these points the water table appears to be standing higher than the flow line in the tile. However, where the water table, as shown in figure 3, was measured at $2\frac{1}{2}$ and at 5 feet from the tile, .it is much lower at the points nearer the tile.

The scale to which it is necessary to reduce the profiles for reproduction in this paper does not permit of the detailed study that the author enjoyed while working with the larger scale original drawings.

It is believed by some,[2] if not by most writers on drainage that water enters a tile line from the bottom as the result of the tile having intercepted a vertical pressure upward. If this view is accepted, a flattened water table curve between tile lines should be the natural consequence. The height above the flow line in the drain, at which the water table stands between drains would be a measure of the magnitude of the upward pressure. If, on the other hand, the theory of a lateral flow into a drain is accepted, a much more rounded curve to the water surface between drains would be expected in order to create sufficient head to cause the lateral movement. In this case, the height of this curve above the flow line in the drain would be a measure of the rate of lateral movement.

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[2] Murphy, D. W.   Drainage engineering, p. 1–172, McGraw-Hill Book Company.   1920.

SUMMARY AND CONCLUSIONS

From the data which were obtained under these widely different soil conditions and widely different spacing and depth of tile, it appears reasonable to conclude that:

1· The water table between lines of tile is practically a straight line, except within a very short distance of the tile.

2. The depth of tile or the spacing between lines of tile does not materially alter the *shape* of the water table.

3. The water table under certain conditions may stand above a tile line at points directly over it and yet the drainage be efficient and the tile lines only partially filled with flowing water.

4. Because of the flatness of the water table, it would appear probable that the major part of the lateral adjustment in the water table, due to the removal of water by a drain, takes place below the flow line; and in that portion of the water table above the flow line the movement is largely vertical. It seems logical that the lateral gradient in the surface of the water table must be greater than has been shown in these profiles before there is a significant lateral movement toward a drain in that portion of the water table which is above the flow line.

5. The depth of tile rather than the spacing between tile lines is the more important feature affecting the efficiency of a drainage system.

6. To obtain the same efficiency (that is the same lowering of the water table) in areas where the vertical pressures differ, the tile must be either deeper or closer together in the case of the greater pressure.

THE ACTION OF PHOMOPSIS CALIFORNICA IN PRODUCING A STEM-END DECAY OF CITRUS FRUITS[1]

MONIR BAHGAT[2]

INTRODUCTION

The aim of this investigation was to study the parasitism of *Phomopsis californica* Fawc., especially as to the manner in which the organism affects the different tissues of citrus fruits.

This fungus is similar to *Phomopsis citri*, which causes stem-end rot and melanose in Florida. *P. californica* was found by Fawcett (1922, 1924, 1926) to be the causal agent of decorticosis (shell bark) of lemon trunks and of a leathery, pliable stem-end rot of citrus fruits.

Of the various citrus fruits inoculated, lemons proved to be the most susceptible to this decay. The stem end, which is the usual place for beginning of decay under natural conditions, was also found to be the ideal place for infection under laboratory conditions. Wounds or punctures always facilitated infection.

It was noted that certain tissues of the lemon fruit were readily invaded by the fungus, while others remained free from invasion. The cells of the loose parenchyma tissues of the inner portion of the rind, known as the albedo, and those forming the axis of the fruit, known as the core, as well as the vascular bundles, were the elements most commonly attacked. On the other hand, the oil-bearing and

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juice-bearing tissues were almost free from the invasion. The effect of the fungus on the fruit was found to be partly mechanical and partly enzymatic.

A study of the enzymes excreted by the fungus during its attack was considered necessary to explain more completely the real nature of the decay.

MICROSCOPIC STUDY OF DISEASED AND SOUND TISSUE

Methods.—Both free-hand sections and material imbedded in paraffin were used. On the whole, it was found that the best fixative was chrom-acetic-urea, composed of 1 per cent each of chromic acid, glacial acetic acid and urea in distilled water. Because of the wax that protects the epidermis of the lemon rind, penetration by the fixing fluid was generally slow. In order to prevent the inner parts of the material from breaking down, in warm weather, before the fixing agent reached them, the material was cut into small pieces and fixed at low temperatures. A period of two days was found necessary for thorough penetration; the renewal of the fixative once or twice during this time seemed to be helpful. To harden the material, especially the diseased lemon rind, it was left in 70 per cent alcohol for three days during dehydration. The material could then be preserved indefinitely in the same strength of alcohol for future use. Of several differentiating stains tested, Magdala red with light green proved to be the best. The mycelial threads became stained a deep pink, while the lemon rind tissues became green.

Structure of the Normal Tissues.—To understand the nature of the decay it was necessary to study in some detail the structure of the normal fruit. The lemon fruit may be considered as a highly modified berry. The mature rind is yellow throughout the external third or half of its thickness, the coloring being due to the presence of carotinoids and oil. The albedo or inner portion of the rind is white in color.

The ovary consists of a single whorl of carpels, the outer layers of which develop the rind and the inner the pulp. The outer layers of the carpels are united along the whole length of their edges to form the rind, while the inner layers fold towards the core of the fruit to form the segments, including their separating membranes (*see* Ross, 1890).

The mature epidermis, which is generally protected with a waxy substance, is composed of one layer of empty, colorless, thin-walled cells mostly rectangular in shape.

In citrus fruits the periderm lies immediately below the epidermis. Next below the periderm is a layer of thin-walled parenchyma cells. Both the parenchyma and the periderm contain many chromatophores. Next below these supporting layers is found the albedo with its loose parenchyma cells extending to the pulp. Between the two parenchyma layers are found more compact parenchyma cells, within which are the widely separated groups of bast fibers.

Any section through the lemon rind reveals three sizes of oil glands, mostly globular in shape. The larger ones are located immediately beneath the small depression of the rind, while the intermediate and small ones are between the large glands. We occasionally found a
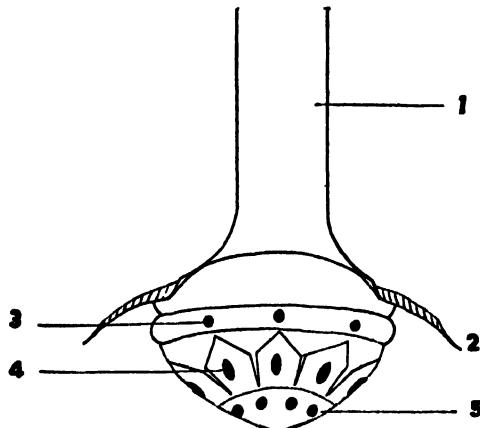
Fig. 1. Diagram of a lemon button; 1, stem; 2, cut calyx; 3, 4, and 5, projecting strands of the three circles representing the branching of the main group of vascular bundles of the stem.

fourth type of oil gland, which resembles a long-necked flask. Each type of gland originates from a mother cell, which divides rapidly. The thin walls of most of the middle cells are soon dissolved, thus enlarging the gland cavity. The protective wall of the oil gland is composed of closely compacted cells without intercellular spaces.

The wall of each segment is enclosed by a narrow, white sheath of parenchyma cells, which connects the albedo tissue with the core. Within the sheath are numerous vesicles, which contain the juice in large, thin-walled cells. The walls of the vesicles are thin and are composed of narrow, elongated cells arranged perpendicularly to the axis of the fruit. Each vesicle is attached to the concave side of its segment wall by a rather delicate stalk. There is much variation in the size and shape of the vesicles, which is due to position and pressure.

In studying the vascular bundles of the fruit it was found that as those of a twig diverge to form the main veins of the leaf, so do the vascular strands branch out to form the fibro-vascular strands of the system of coalesced carpels called the fruit. This divergence takes place in the receptacle, which is united with the persistent fleshy calyx to form the button of the fruit. Examination of the lower surface of a normal button where it is attached to the fruit reveals to the unaided eye three concentric circles of tooth-like projections (3, 4, and 5 in fig. 1). The button scar (the depression from which the button has been removed) shows the continuation of these three

Fig. 2. A longitudinal and a cross-section of mature healthy lemons, with the vascular-bundle strands inked to show their distribution in the rind and core.

circles of bundles. As long as the button is attached to the fruit, the strands projecting from the button will be connected with the corresponding ones in the fruit. Between the button and the fruit tissues lies the abscission layer at which separation takes place when the button is pulled out. In the longitudinal section of the fruit (fig. 2), the strands of the main central circle are seen to continue downwards from the button into the core and the nearby tissues. The vascular bundles of the second circle of the button, however, diverge and proceed between the rind and the pulp, supplying mainly the latter. The strands of the third circle diverge still more to form a network through the rind. In the cross-section of the fruit (fig 2) most of the vascular strands appear to the naked eye as small specks scattered throughout the rind, between the rind and the segments, and in the core. There is always a main bundle found midway along

the side of each segment where it touches the albedo. This bundle is similar to the midrib of the unmodified lemon leaf. There is also a smaller bundle at the angle between each two segments (fig. 2).

The core is composed mostly of large spongy parenchyma cells, among which *Phomopsis californica* grows readily. In the outer portion of the core, extending from the stem to stylar end, are the strands of the main vascular bundle.

The Diseased Tissue.—The first external symptom induced by placing spores of *Phomopsis californica* in the button scar of mature lemons is the development of a characteristic pliable leathery circle just around the button. During the first week of development this circle enlarges gradually but slowly towards the stylar end without any visible discoloration. Later the invaded surface loses its natural color and becomes honey yellow, then ochraceous buff, and finally buff brown. Such changes vary somewhat with differences in temperature, humidity, and age of fruit. When the discoloration covers nearly one-fourth the area of the rind, a water-soaked belt can be seen advancing ahead of the line of discoloration. This belt is not sunken and its advancing margin is the border-line between the diseased and healthy tissues. This belt, the width of which varies from one-fourth to one-half inch, encircles the rind in a continuous but wavy form. It continues to advance towards the stylar end, followed immediately by pliability and discoloration, the entire surface of the fruit becoming buff brown in color. In some cases, under favorable conditions, a furrow-like depression opposite each segment of the fruit is an added symptom.

When spores of *Phomopsis californica* are sown in distilled water upon the rind of a sound lemon fruit, no infection ordinarily results.

In an experiment in which drops of water containing spores were placed on the uninjured surface of the rind most of the spores germinated, but the germ tubes failed to penetrate the rind. In experiments in which wounds were made through the outer oil-bearing tissue of the rind, however, infection took place and development of decay followed. Such decay took approximately three weeks to become visible on the surface of the rind. Once the hyphae reached,. by means of wounds, the inner tissue of the rind, they progressed without difficulty. On the other hand, if the button scar was filled with the spore suspension, either in water or in nutrient solutions, no further wound was necessary to cause infection. A puncture or a drop of prune juice, however, hastened infection. The button scar evidently furnishes ideal conditions for the development and penetration of the fungus.

An examination of various tissues after the development of decay indicated that the fungus was not present in all parts of the affected portion. This was shown by transfers to glucose-potato-agar plates from the different zones of diseased lemons. For example, five transfers were made from a lemon (fig. 3) each consisting of nearly the same quantity of tissues taken approximately 2 mm below the epidermis. The five plates were incubated for a week under a bell jar at room temperature. Transfer 5, made from the sound area, and transfer 4, made from the water-soaked advancing zone, showed no
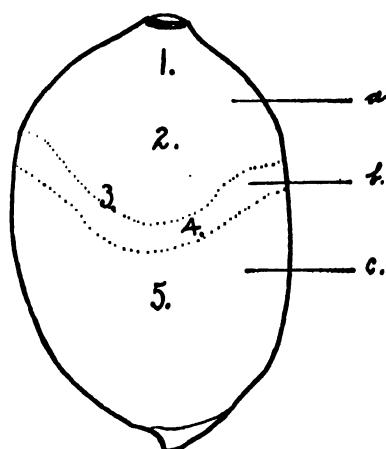
Fig. 3. Diagram of a lemon attacked by *Phomopsis californica: a,* discolored diseased rind; *b,* water-soaked advancing zone; *c,* healthy rind; 1 to 5, points of isolation (see text, page 158).

growth. Transfer 3, made from the advancing margin of discoloration, gave an excellent growth. Transfer 2, taken a little farther back, also gave a fair growth, while transfer 1, which was still farther back, gave no growth. This experiment was repeated three times with similar results. This result indicated that the advancing zone at 4 was free from the organism, that the margin of discoloration at 3 had the most active mycelium, that the tissue at 2, farther back, had less active mycelium, and that the tissue still farther back, at 1, had no live mycelium. As judged from microscopic examination of tissues at 1, showing the absence of mycelium, it is inferred that the mycelium had been dissolved.

When a diseased fruit was cut longitudinally, the internal pathological changes seemed to coincide, to a considerable extent, with the external symptoms (fig. 4).

The three zones, namely, the discolored, the water-soaked, and the healthy, could be recognized. The discoloration in the core advanced far ahead of the limit found in other tissues. In the rind the discoloration appeared to travel faster through the albedo than through the outer rind; the discoloration in the albedo was always in advance of the discoloration upon the surface. In longitudinal sections the more rapid advance in the albedo produced a tongue of discolored tissue projecting between the sound pulp and the sound outer rind.
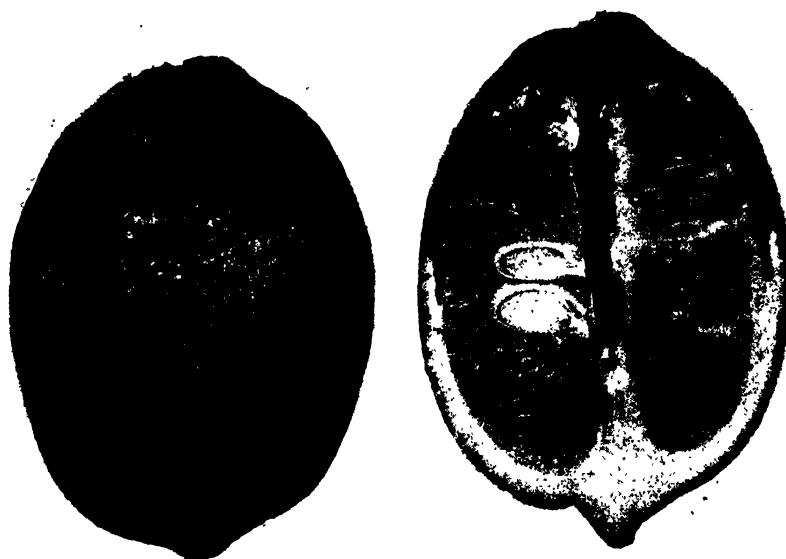
Fig. 4. External and internal symptoms of *Phomopsis californica* on a lemon fruit showing slightly affected pulp, advancing tongue of decay in albedo, and the decayed core.

This continued until the whole rind was entirely discolored. The pulp tissues seemed to be the least affected. Only in advanced cases did the walls of the segments and the walls of the vesicles next to them become discolored. The outer rind and the pulp tissues were usually firm. The parenchyma cells of the albedo, when severely affected, tended to become separated.

With the aid of a microscope, it became evident that the advancing water-soaked area was free from the mycelium. The pathological changes in a cross-section of the core just ahead of discoloration seemed to be mainly plasmolysis and maceration (fig. 5). Further examination of the slides revealed that the epidermis, the oil-bearing

tissues, and the vesicles containing the juice, were also free from the organism. No hyphae were found inside or in the neighborhood of epidermal cells, oil glands, or juice vesicles. The attack of the fungus seemed to be mainly confined to the spongy parenchyma cells of the core and the albedo. In such tissues bits of the mycelium were frequently seen inside the macerated cells, or along their cell walls. In later stages of decay, invasion of the vascular bundles in the core and the albedo was noted, but it was evident that the hyphae were most abundant in the parenchyma tissues surrounding these bundles. The attacked cells, besides being separated from each other along the middle lamellae, become plasmolyzed. Occasionally the walls of
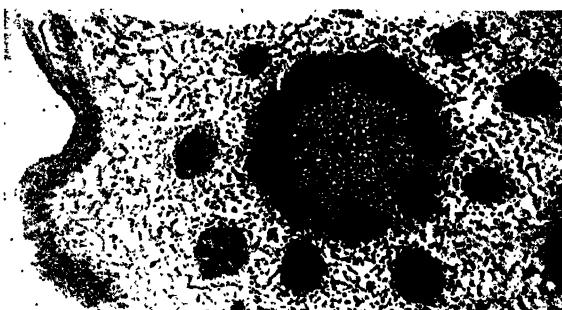
Fig. 5. Cross-section through a core 2 mm beyond discoloration, showing mainly plasmolysis and maceration.

the penetrated cells were broken in one or two places. Where the mycelium came in contact with the walls, the latter decreased ·in thickness. When sections were made from rind which had been discolored for a considerable time, such as that close to the stem end of a half-decayed fruit, the mycelium was found to be dissolved. On the other hand, when sections were prepared from the newly attacked tissues, especially from those close to the water-soaked area, long mycelial threads could easily be detected.

The fungus exhibited a very strong deamidase reaction, as will be shown in the section on fungus enzymes. The large amount of ammonia formed in the process suggested that this substance might have some connection with the development of the water-soaked belt in the decaying fruit. Accordingly a preliminary test was made for the purpose of observing the effect of ammonia on sound fruit. A lemon was cut twice longitudinally at right angles through the core and once transversely, making eight equal portions. These were placed separately in bottles containing 4, 2, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ and $\frac{1}{32}$ per cent solutions of 28 per cent ammonia (sp. gr. 0.9) respec-

tively. All the flasks were corked tightly and were left at room temperature for observation. The first three solutions caused the rind to become dark brown in less than six hours, while the rest showed moderate effects. Solutions of ½ and ¼ per cent developed on the rind the typical buff-brown color of stem-end decay, and caused maceration in both the albedo and the parenchyma cells of the core. The vesicles also were separated from each other. The last three solutions caused no change in color of the mature rind, but caused slow disintegration.

In another experiment solutions of 1 per cent, and one-half and one-fourth of 1 per cent of ammonia were drawn inside healthy mature fruits by means of a suction pump. A piece 3 mm in thickness was cut from the stylar end of a lemon and this end was fitted tightly in the neck of a suction flask. The button of the stem end was then removed, the exposed end wounded once with a scalpel, and the ammonia solution applied. A partial vacuum of ten pounds quickly drew the solution into the lemon. In this case even a 1 per cent solution developed internal and external symptoms similar to those caused by the disease. This would suggest the possibility that the water-soaked effect and the discoloration which follows the water soaking may be due in part to the by-products of enzymatic action, such as ammonia.

The discovery of the mycelium within and along the vascular bundles led to the following experiment. Six lemons, each with a stem one-half inch in length, were placed perpendicularly in such a position that the ends of their attached stems were immersed separately in a suspension of *Phomopsis* spores. In another six the attached stems were longitudinally split, and one-half of each was removed with its respective half of the button. These were treated in the same manner. This experiment covered a period of three weeks at room temperature, more of the suspension being added occasionally to the containers so that the cut ends were always in contact with the liquid. In those fruits which had normal stems the external symptoms of the disease developed gradually until the entire rind was discolored. In the fruits of the second set the symptoms appeared only on the sides which had connection with the suspension through the half stems. When samples of fruit were cut longitudinally, the internal pathological changes agreed with the external symptoms. Culture tests showed that only the discolored half contained the fungus, indicating that the mycelium advanced into the fruit along the vascular bundles on the half of the stem not cut away, from which it freely invaded the nearby tissues.

RELATION OF THE KINDS OF TISSUE AND THEIR
CONTENTS TO THE GROWTH OF THE FUNGUS

Oil-bearing Tissues.—It was shown above that the *Phomopsis* hyphae did not at first enter the oil-bearing tissues, but concentrated in the core and in the albedo. These facts led to the following experiments to determine whether the lemon oil itself might have anything to do with the absence of hyphae in the oil-bearing tissues.

The rind of a fresh healthy lemon was removed and was cut into longitudinal strips one-fourth inch in width. These strips were again cut so that the albedo layers were severed, as far as possible, from the external rind. The inner and outer strips were then placed separately in culture tubes, at the bottom of which moistened pieces of cotton had been previously inserted. All the tubes were then autoclaved and inoculated with the organism. In two weeks good growth of the fungus was observed in each tube, regardless of the portion of the rind use.

In a similar experiment, the same two parts of the rind were placed in culture tubes under as sterile conditions as possible and inoculated as before. Great care was taken to have the media sterile without applying heat. All the inoculated tubes of albedo developed an excellent growth of *Phomopsis* while the corresponding inoculated tubes of outer rind showed no growth. The tissues of the albedo first became brown in color, then were nearly covered with mycelium, and finally were dotted with pycnidia. The inoculated tubes containing the outer rind showed no growth of any kind, remaining the same as those not inoculated.

In a third experiment the fungus failed to grow when 1 per cent of pure lemon oil was added to a carbon-free medium; this indicates that the fungus was unable to utilize the oil as a sole source of carbon.

The last result led to a fourth experiment in which 1 per cent of lemon oil was added to a full nutrient solution in flasks and inoculated. The flasks were frequently shaken so that the oil was kept mixed with the other nutrients for a considerable time. No growth took place in these cultures, but very good growth occurred in check flasks of the same medium which had received no oil. In another trial strips of albedo and oil-bearing tissue were prepared as in the second experiment and pure lemon oil was added to the strips before inoculation. No growth took place in any of the tubes. The albedo, which

was normally a good medium for the fungus, failed to support growth on the addition of the lemon oil.

It therefore appeared probable that the oil-bearing tissues of a normal rind are protected by the oil from invasion by the fungus, which could thrive well on tissues free from the oil, such as the core and the albedo. In the first experiment, the fungus also grew well on the oil-bearing portion of the rind after being autoclaved, probably because the oil had been volatilized by heat.

The Juice-bearing Tissue.—During the present investigation a considerable number of lemon fruits attacked by *Phomopsis californica* were examined, in both early and advanced stages. To the naked eye the pulp of these fruits seemed to escape the attack, especially in the early stages. When examined microscopically, however, the walls of the segments were found to be infected, while the walls of the vesicles within were usually free from invasion. Only in the very advanced stages were the vesicular walls attacked. Even in such advanced cases the mycelia were found in the walls of the vesicles, but never in the juice itself. This last observation regarding the juice was similar to that regarding the oil and suggested further experiments.

Pulp of a sound lemon from which the core had been removed was squeezed in cheesecloth and washed several times with water until it was freed of nearly all its natural juice. This pulp supported a very good growth when it was inoculated with the *Phomopsis* organism, while untreated pulp with all the juice left in it permitted only a very poor growth.

In another experiment, no growth was obtained when lemon juice was used as a medium, while both orange juice and prune juice gave an excellent growth. It was found later that the spores of *Phomopsis californica* were unable to germinate in undiluted lemon juice; but when the latter was diluted 1 to 1 a few spores gave rise to germ tubes.

When citric acid was added to a full nutrient solution at the rate of 4 per cent, it inhibited the growth of the fungus.

In another experiment, six flasks with 50 cc of carbon-free nutrient solution containing 1 per cent tannic, malic, oxalic, citric, formic, and tartaric acids, respectively, were inoculated with a *Phomopsis* pycnidium.

In the first few weeks a slight growth appeared in the flask containing the tannic acid. Later the solution turned brown and then black, and finally a good growth developed with pycnidia formed over a solid mat of mycelium. In the malic acid medium a fair growth was obtained, but the pycnidia were lacking. In the oxalic acid a

poor growth developed. In the citric, formic, and tartaric acids, however, no growth took place.

Camp (1923) found that none of the fungi (including *Phomopsis citri*) which commonly attack citrus fruits, could thrive on citrate as the sole source of carbon.

Since even the addition of citric acid to full nutrient solutions checked the growth of the fungus, it seems highly probable that the acid is responsible for the protection of the juice-bearing tissues from invasion.

THE ENZYMES PRODUCED BY THE FUNGUS AND THEIR RELATION TO THE DECAY

The important rôle played by enzymes in plant-disease phenomena has long been recognized and studied, and the subject is ably summarized in the recent papers of Hawkins and Harvey (1919) and Harter and Weimer (1921). In the course of the investigations on the *Phomopsis* rot it became evident that a survey of the enzymes of the fungus was necessary to help elucidate the nature of the parasitism. Accordingly, after a preliminary test for the presence of enzymes in an extract made from the fungus, the enzymes thought to be most directly concerned in the intimate parasite-host relationship were tested for.

Culture Media.—As a liquid medium, Duggar's (-1909) standard nutrient solution was prepared with the addition of a small quantity of sodium chlorid as follows:

Ammonium nitrate	1.0 gram
Dihydrogen potassium phosphate	0.5 gram
Iron chlorid	trace
Sodium chlorid	trace
Magnesium sulfate	0.25 gram
Cane sugar	5.0 grams
Water	100.0 cc

Cane sugar was omitted when a non-carbon medium was needed and 1.5 per cent of "Bacto" agar was added when a solid medium was required.

A solid non-carbon medium, which did not support the growth of microorganisms, was prepared in large quantities and is referred to as "stock medium" in the following discussion.

. *The Preparation of the Mycelial Extract and Mycelial Powder.*—
Pure cultures of *Phomopsis californica* were grown in Czapek's nutri-

ent solution in large flasks. In two months thick mats were developed. These were washed gently with distilled water, and ground in a porcelain mortar with approximately an equal weight of clean quartz sand. The resulting fine pulp with an equal volume of sterile water was transferred to a 500 cc Erlenmeyer flask, a few drops of toluene
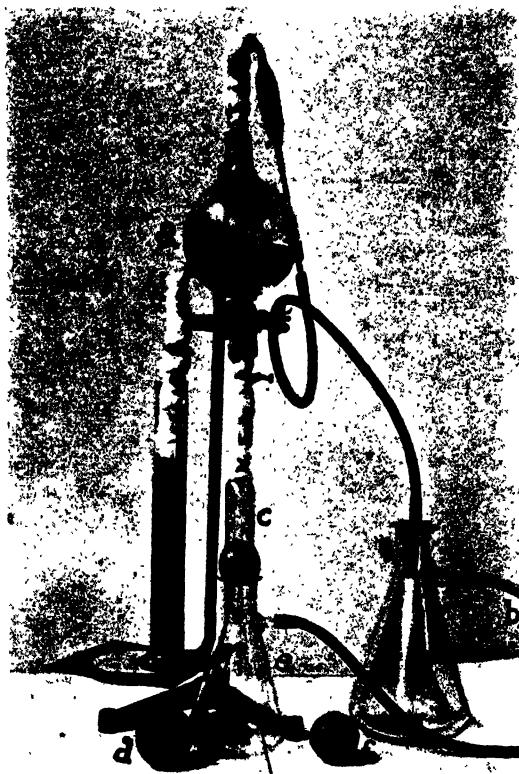
Fig. 6. Filter apparatus used in testing the effects of the mycelial extracts on the healthy tissues of the lemon. In this apparatus the liquid is drawn up through the Mandle filter candle into the large flask (*a*) under five pounds vacuum at *b*, which is connected to a water-vacuum pump. The sterilized filtrate runs off below by means of a stop-cock and the outlet glass tube, which is enveloped in the larger tube, *c*. The only possibility of contamination is from the air. The connecting tubes are wrapped with thick layers of cotton as a precaution against such contamination. Before using, the apparatus is sterilized in an autoclave; *d*, lemon ready to be treated; *e*, filter flask connected with a water-vacuum pump; *f*, lemon with exposed vascular bundles of the fruit after the removal of three millimeters from the stylar end to facilitate suction.

added, and the whole incubated at 30 degrees C for several hours. From time to time the flask was vigorously shaken, and finally the material was allowed to settle. The supernatant liquid was siphoned

off and filtered through a filter paper, and then sterilized by drawing it through a Mandler filter candle under five pounds partial vacuum (fig. 6). The apparatus used was an imitation of the one described by R. E. Smith (1917). The apparatus with the sterile mycelial extract was stored in the culture room away from the bright light.

Mycelial powder was also prepared from thick mats of the fungus by the Acetone-Dauer-Hefe method of Albert, Buchner, and Rapp (1902) with a few modifications. The mats were ground in a mortar to a fine pulp, four times their volume of acetone was added, and the whole stirred five minutes. The material was drained as dry as possible on a suction filter and the process was repeated, using a quantity of acetone equal to that of the mycelial pulp. The mycelium was finally similarly treated with an equal volume of ethyl ether and spread out to dry for 12 hours in an oven at 30° C. The desiccated material was then ground to a fine powder and stored in a brown glass bottle.

Effect of the Mycelial Extract on Sound Lemons.—Early during this study strips from the rind were placed in the extract for observation, but later the entire fruit was used, the extract being placed in a puncture 1 inch deep made through the stem end. By the method described in connection with the experiments on the effect of ammonia, definite quantities of the extract were drawn into fruit. When the active extract was used, the sterilizing apparatus was employed in such a way that one drop at a time filled the button scar of the fruit (fig. 6). As checks, some extract inactivated by boiling, and some distilled water were drawn into other fruits.

TABLE 1

Effect of Pulling Mycelial Extract into Fruit Under Pressure

Quantities	Active extract	Boiled extract	Distilled water
2 drops	Very slight effect	No effect	No effect
5 drops	Much effect	No effect	No effect
10 drops	Typical disease symptoms	No effect	No effect

Many ripe lemons were first treated externally by dipping in 5 per cent solution of sodium borate for one minute. After a few days 27 fruits were selected from those which seemed normal and 3 fruits each were treated by the methods shown in table 1. After such treatment all the fruits were incubated at 30° C for three days.

After the incubation period each fruit was examined carefully, and then was cut longitudinally for study of the effect on the internal tissues.

The first three fruits, treated with 2 drops of active extract, showed only very slight effect. The core tissues were affected more than the rest and no external changes were visible. The second three fruits, with 5 drops of active extract, developed to some extent internal and external changes which resembled those of an early attack of the disease. The next three fruits, which received 10 drops each, showed characteristic symptoms both internal and external. The epidermis, however, developed a water-soaked appearance instead of the usual buff brown. This condition was almost typical of that of the advancing zone that appears ahead of the discoloration in tissues attacked by the fungus, as previously described. Although these symptoms appeared in a shorter time than with fungus inoculations, the general disintegration among the internal tissues, and the leathery pliable condition of the surface was practically the same as in fruits inoculated with the fungus. When the vascular bundle strands were traced down through the tissues, it was noticed that they were darker in color than the adjacent tissues. The tissues adjacent to the bundles were more affected than any of the other portions. The next nine fruits, which received the boiled extract, did not show any of the changes described, regardless of the quantities drawn into them. The fruits which received the distilled water appeared normal in every respect.

It was evident, therefore, that the mycelial extract was capable of developing some of the characteristic symptoms of the disease, and that the amount used had a direct relation to the degree of the development of the symptoms.

Testing for Cytase.—The breaking-down of cellulose by microorganisms has been studied by Kellerman and McBeth (1912), McBeth and Scales (1913), Cooley (1914), and many others.

With *Phomopsis californica* the following experiments were carried out: Swedish filter paper was soaked in the carbon-free nutrient solution and inoculated with pycnidia in six petri dishes. Good growth took place, showing that the fungus was able to utilize the cellulose of the filter paper as the only source of carbon.

To obtain more accurate results, pure cellulose precipitate was prepared as described by Cooley (1914) and added to the stock medium. The resulting solid cellulose medium was then autoclaved, plated, and inoculated with pycnidia. The good growth that resulted was further evidence of the ability of the fungus to attack cellulose.

In another experiment 5 cc of the mycelial extract was added to tubes of cellulose medium along with a few drops of toluene for disinfection. As a check, boiled extract was used in tubes in the same way. The cellulose agar columns in those tubes receiving the active extract were cleared to an average depth of 4 mm, while those of the check tubes receiving boiled extract remained uniformly cloudy.

The experiments above, as well as the microscopical observations previously described, showed the ability of the fungus to attack celluloses, and indicated that the enzyme cytase was present in the mycelial extract.

Testing for Pectase and Pectinase.—The enzyme which is capable of hydrolyzing pectic compounds of the cell wall is called pectinase (pectosinase by some investigators), while the one causing their coagulation is termed pectase. Since it was found in miscroscopical examinations that the middle lamellae, especially those of the albedo cells, were dissolved, the demonstration of pectase and pectinase was attempted experimentally.

A commercial preparation of pectin (Certo) as a substrate gave negative results for pectase when tested with both the mycelial extract and the powder; it was not coagulated by either. In demonstrating the actions of pectinase many investigators have worked principally with mycelial extracts. In this investigation not only the mycelial extract but also the liquids in which the fungus had grown were tested for this enzyme.

Further experiments were conducted to determine the macerating power, or rather the pectinase action, on four different tissues. Both the mycelial extract and the powdered mycelium were used. Discs of lemon rind, potato, carrot, and beet were cut into uniform thickness and shape (17 mm in diameter and 5 mm thick) by means of a cork borer and a knife. In each of twenty 150 cc Erlenmeyer flasks were placed two discs of one of the four substrata, a few drops of toluene and 25 cc of the mycelial extract or water. Five flasks were used for each substratum, two containing the active extract, two the boiled extract, and a fifth distilled water. The flasks were kept at 30° C.

Only the discs in the active, unheated extract showed evidence of maceration. The extent of maceration was judged by the relative ease of rupture of the discs by means of dissecting needles, and by the numbers of separated cells revealed by the microscope. The beet discs were completely disintegrated in 48 hours, and the anthocyan disappeared in 24 hours. This indicated that the active extract not only destroyed the middle lamella, but also made the protoplasts more

permeable and destroyed the pigment that exosmosed. While the boiled extract caused no maceration or destruction of the pigment, it did permit diffusion of the pigment into the surrounding water. The carrot discs in the active extract showed only slight maceration in 48 hours, and this only on the perimetrical part. The enzyme attacked the potato discs very vigorously and the cells showed complete separation in 24 hours. Lemon-rind discs resisted dissolution, exhibiting but a slight effect at the perimeter. This work was repeated, using the culture medium in which fungus had grown. The results were similar to those with the extract; that is, maceration was effected only by the unheated material. The production of pectinase by *Phomopsis californica* was therefore demonstrated.

Testing for Invertase and Maltase.—Since disaccharides as well as cellulose are important constituents of lemon rind, as pointed out by Bartholomew and Robbins (1926), it was thought advisable to test for invertase and maltase also.

One gram of pure sucrose was added to 100 cc of the stock medium as the sole source of carbon. To another 100 cc of the stock medium one gram of pure glucose was added as the sole source of carbon. Petri dishes with both media were inoculated with a few spores of the fungus. In one week growth in the glucose cultures was much ahead of that in the sucrose plates. Gradually, however, the development of the fungus in the sucrose plates became more vigorous, and the final results were similar to those with the glucose plates. This sort of development gave an indication that it was necessary to invert the sucrose into glucose and fructose before it became available.

In a second experiment 5 cc of the mycelial extract was added to 50 cc of a 1-per-cent sucrose solution with a few drops of toluene as an antiseptic. As a control, boiled mycelial extract was used in the same manner. A duplicate set was made. After 24 hours at 30° C the reducing power of each solution was determined. Use was made of the rapid volumetric method of titrating the unknown directly against Fehling's solution, 10 cc of which was considered to be completely reduced by 0.05 gram of glucose (Haas and Hill, 1917). Table 2 shows the results obtained.

These experiments showed that *Phomopsis californica* was able to produce the enzyme invertase.

The presence of maltase was demonstrated in a way similar to that of invertase. The growth of the fungus was rather slow at first, but eventually became vigorous, indicating that the organism hydrolyzes the disaccharide to dextrose before assimilating it.

In a second experiment 25 cc of a 1 per cent maltose solution was treated with 2 cc of mycelial extract and a few drops of toluene added as an antiseptic. As a control both the boiled extract and distilled water were added to another set of flasks, each of which contained

TABLE 2

QUANTITATIVE STUDY OF THE INVERTASE IN THE MYCELIAL EXTRACT

No.	Myc. extract added to 50 cc of 1 per cent sucrose solution	Substratum used to reduce 10 cc of Fehling's	Glucose in substratum	Glucose due to invertase
	cc	*cc*	*per cent*	*net per cent*
1	5	5.6	0.9	0.70
2	5 (boiled)	25.0	0.2	
3	5	5.8	0.86	0.66
4	5 (boiled)	25.0	0.2	

the same amounts of sugar solution and antiseptic. After 48 hours at 30° C. the reducing power of each solution was determined by direct titration against 10 cc of Fehling's solution as shown in table 3.

The above experiments showed that *Phomopsis californica* had the ability to produce maltase.

Testing for Emulsin.—Emulsin was discovered in *Aspergillus niger* by Bourquelot (1893) who also discovered it in 25 species of higher fungi. The presence of emulsin in *Phomopsis* mycelia was determined in a preliminary way by the ability of the fungus to use the glucoside arbutin. Owing to the solubility of glucosides, the agar containing them is clear and the action of the enzymes could be easily detected. Arbutin, if hydrolyzed, yields glucose and hydroquinone, and the latter stains the clear medium with a brown color.

TABLE 3

QUANTITATIVE STUDY OF THE MALTASE IN THE MYCELIAL EXTRACT

No.	Amt. of myc. extract added to 25 cc of 1% maltose solution	Amt. of substratum used to reduce 10 cc of Fehling's	Increase in reducing power due to maltase
	cc	*cc*	*per cent*
1	2	5.0	62
2	2 (boiled)	8.1	
3	2 distilled, H_2O	8.2	

Arbutin added to the stock medium at the rate of 1 per cent supported an abundant growth of the organism. A brown stain soon appeared in this medium, indicating hydrolysis of the glucoside and the presence of the enzyme emulsin.

In a second experiment 5 cc each of the liquid in which the fungus had grown was added to a set of 5 tubes of arbutin medium. As a check, 5 cc of the boiled solution was added to another set. In the first set only were the agar columns stained brown, indicating that the arbutin had been hydrolyzed to glucose and hydroquinone.

A third experiment was conducted in which the mycelial powder previously described was employed in a thin layer over the surface of each arbutin-agar column. As a check some of the powder was inactivated by heat before use. The brown color, due to hydroquinone, was found to diffuse down to the bottom of the columns in the tubes treated with unheated mycelial extract, while the checks remained unchanged.

A fourth experiment, in which the activity of emulsion was estimated by the determination of the reducing power of the hydrolytic products, was conducted as follows: In each of 4 Erlenmeyer flasks was placed 25 cc of 1-per-cent arbutin solution. Also to each flask 0.25 gram of the mycelial powder and a few drops of toluene were added. The powder of the third and fourth flasks was inactivated by heat before use. The four flasks were shaken vigorously for one minute. In three days at 30° C the solutions in the first two flasks turned brown, while those of the other two remained clear.

It required only 5.4 and 5.6 cc respectively of the solution from the first two flasks, to reduce 10 cc of Fehling's solution, while more than 25 cc of the substrate of the last two flasks were required to cause the same action. This experiment indicated that *Phomopsis californica* had both intracellular and extracellular emulsin.

Testing for Inulase.—A 5 cc portion of mycelial extract and a small amount of mycelial powder was added respectively to 25 cc quantities of 1-per-cent inulin solution in Erlenmeyer flasks. After an incubation period of 3 days at 30° C the materials were tested for reducing sugars. All failed to reduce Fehling's solution, proving that *Phomopsis californica* does not produce the enzyme inulase under the conditions used.

Testing for Proteases.—As protoplasm consists largely of proteins these occur in all plants. Filamentous fungi, especially *Aspergillus* and *Penicillium,* have been commonly used to demonstrate proteoclastic reactions. Bourquelot and Herissey (1895) were the first to discover the protein-digesting enzymes in *Polyporus sulphurous.*

With *Phomopsis californica* experiments were conducted, using fibrin, gelatin, casein, and albumen as substrata. Powdered blood fibrin was added to the stock medium in the ratio of 1 per cent. Care was taken to shake the medium while pouring so that the protein

particles would be as evenly distributed as possible. Good growth took place on petri dishes of this medium, indicating that the fungus was able to utilize the fibrin as the only source of carbon, breaking it down with the aid of protease.

In another experiment the mycelial powder and red fibrin were used according to the method described by Reed (1913), in which the fibrin grains were stained in 1-per-cent Congo red and the color fixed by immersion in boiling water. The proteolysis was measured then by the amount of stain liberated into the solution.

The mycelial powder in neutral and alkaline solutions containing 20 cc of water and 2 grams of red fibrin showed a greater amount of red color than in an acid solution. The powder after being heated gave no color under the same conditions.

In a third experiment Rumbold's (1908) gelatin in plates was inoculated with fresh mycelia. After six days a good growth was noticed, with a liquefied circle 1 inch in diameter indicating a protease reaction.

In a fourth experiment the presence of erepsin was demonstrated by its hydrolytic action upon 1-per-cent casein in the stock medium. Good growth resulted in a week after inoculation with mycelium, and the secretion of erepsin was so uniform and abundant that a wide band of dissolved casein enclosed the margin of advancing hyphae on petri dishes.

Egg-albumin-agar prepared as recommended by Reed (1913) was used to test for the production of trypsin. Good growth, similar to that on the casein medium, took place, and a halo of clear agar formed ahead of the advancing mycelia; this shows that *Phomopsis californica* excretes trypsin.

Testing for Amidases.—Amidase is the enzyme which hydrolyzes acid amides to ammonia and organic acids. Accordingly, qualitative and quantitative tests for ammonia in the substrate, after incubation with the fungus or its enzyme preparations, were employed to demonstrate the presence of amidase. Since the ammonia formed produces an alkaline reaction which completely dominates the small concentration of H-ions contributed by the weakly ionized organic acids, any indicator which gives a brilliant color in the alkaline range may be used in the qualitative test. Rosolic acid, as recommended by Crabill and Reed (1915) was used; this gives a brilliant red color above pH 8.

. In stock solution with 1 gram of asparagin and 1 cc of 2-per-cent rosolic acid in 100 cc of solution, kept at 20 to 25 degrees C, the

fungus grew slowly during the first few days, but during the second week abundant mycelia were developed, covering a circular area 1 inch in diameter. The rosolic acid, which gave the medium a yellow tint, turned red during the first day when the asparagin was attacked by the *Phomopsis* mycelia. During the second day the red color diffused readily all around and ahead of the advancing hyphae. During the third day there was no trace left of the yellow color in any of the transfers. In the controls, however, where no asparagin was added to the stock medium, no growth took place, and the yellow color of the rosolic acid was unchanged.

The above experiment was repeated, using in this case the mycelial powder, which was placed at the center of each petri dish. In an hour the unheated powder turned the yellow medium red, while in a similar test heated powder showed no effect.

In a third experiment mycelial extract was placed in shallow wells 2 mm in diameter and 3 mm deep. At the end of one-half hour at 30° C the cherry-red color diffused all around the surfaces of each well, and the red color invaded all the area of each plate in a day or two. Similar wells in the medium filled with the extract after it had been boiled gave no red color.

In a fourth experiment twelve test tubes of rosolic-acid medium were treated in three different ways. The first four tubes were each inoculated with a pycnidium. The pycnidia of the third and fourth tubes were heated over the flame before use. To each of the fifth, sixth, seventh, and eighth tubes 0.1 gram of the mycelial powder was distributed over the exposed surface. The powder of the seventh and eighth tubes was heated. To each of the remaining four tubes 5 cc of the mycelial extract was added; the extract of the last two tubes was boiled before use. After one week at 25° C the six controls, to which the heated materials were added, gave negative results, but the other six tubes gave positive results in different proportions. The red color diffused to the bottom of the two test tubes having the mycelial extract, while it diffused to a depth of only 2½ cm in the case of the pycnidia, and to twice as much in case of the mycelial powder.

A fifth experiment to measure the amount of ammonia liberated by the enzyme amidase was conducted as follows: to 25 cc of a 1-percent solution of asparagin in a 250-cc Erlenmeyer flask were added 25 grams of mycelial powder and 1 cc of toluene. As a control, the fungus powder used in this experiment was first autoclaved. Both the controls and the flasks with the active enzyme were run in dupli-

cate. After 24 hours at 30° C, the contents of each flask was filtered through a filter paper. Ten cc of each of the four filtrates was transferred to four test-tubes, and the ammonia was determined by the aeration method of Folin, with the tubes connected in series, so that four aerations could be made at the same time. Folin tubes with perforated bulbs were used for aerating the media and for delivery of the NH_3 into each receiving tube containing 50 cc of 4-per-cent boric acid. To prevent excessive foaming two drops of kerosene were added to each of the four tubes containing the 10 cc of medium. The medium was made strongly alkaline to phenolphthalein by the addition of one drop of a saturated NaOH solution just before connecting each tube with its receiving tube containing the boric acid. A filter pump was connected to the last receiving tube and the solutions were aerated for 24 hours at the rate of approximately 50 liters of air an hour. The receiving tubes were titrated directly against standard sulphuric acid, brom-phenol-blue being used as an indicator. Table 4 shows the results obtained.

These experiments showed that *Phomopsis californica* possessed intracellular and extracellular amidase. ·

TABLE 4

AMMONIA PRODUCED BY THE ACTION OF AMIDASE OF PHOMOPSIS CALIFORNICA
UPON ASPARAGIN

Flask	·	NH₃ liberated
1	25 cc of 1-per cent asparagin and 0.25 gram of mycelial powder...	2. 26 mg
2	Same as flask 1..	2. 10 mg
3	Same as above but the powder inactivated............................	0. 93 mg
4	Same as flask 3..	0. 97 mg

Testing for Lipase.—Oil being a component of the lemon rind, it was thought advisable to test for lipoclastic ability in the fungus. First, use was made of litmus-cream-agar prepared, as recommended by Crabill and Reed (1915), by adding 10 cc of cream to 50 cc of distilled water and 40 cc of 10-per-cent gelatin solution. Blue litmus solution was then added to give the medium a deep blue color. The material was steamed one-half hour and then cooled and inoculated with mycelium of *Phomopsis*. After an incubation of one week the litmus medium remained blue, indicating at least that an insufficient quantity of fatty acids had been formed to raise the H-ion concentration of the medium to pH 5.5, the point at which the indicator turns pink.

This qualitative test was followed by quantitative tests in which 1-per-cent lemon-oil emulsion, methyl acetate, and ethyl acetate were used as substrates.

Fifteen cubic centimeters of each medium was used and 0.1 gram of the mycelial powder was added. Toluene was used as antiseptic. For the checks the power was inactivated by heat before use. After the incubation period the material of each flask was filtered and the acidity was titrated with N/10 NaOH, using phenolphthalein as an indicator. The average results of the duplicate experiment are given in table 5.

TABLE 5

Lypolytic Action of the Mycelial Powder

Flask	Substrate	Mycelial powder	NaOH to neutralize 10 cc substrate
			cc
1	Methyl acetate	Active	5.3
2	Methyl acetate	Inactivated	0.2
3	Ethyl acetate	Active	5.75
4	Ethyl acetate	Inactivated	0.2
5	Lemon-oil emulsion	Active	0.45
6	Lemon-oil emulsion	Inactivated	0.1

The above experiments showed that *Phomopsis californica* did not exhibit any extracellular lipase, yet it seemed to contain an intracellular one. This was demonstrated chiefly by the inability of the fungus to grow on or use oils, while its mycelial powder showed a lipolytic action, as seen in table 5.

DISCUSSION AND CONCLUSIONS

As described in the foregoing pages, the water-soaked belt ahead of the discoloration on the diseased fruit was always found to be free from mycelium of the causal organism. It is probable that some soluble substance produced by the fungus diffuses through the tissues and kills the host cells some distance ahead of the mycelium. The pathological changes in the advancing belt are mainly plasmolysis and maceration. Both the mycelial extract and the liquid in which the fungus had grown developed considerable enzymatic activity. Healthy tissues when acted upon by these liquids showed typical characteristic symptoms of the disease. These liquids when boiled, however, lost their ability to produce any of the symptoms of disease.

The fact that the mycelium, which is abundant at the margin of discoloration in the affected rind, later breaks down and dissolves when it is left far behind the advancing margin, and disappears entirely in old affected tissues, led to the conclusion that the enzymes and their by-products are probably able to dissolve these older hyphae.

In regard to the manner of invasion, it was shown that the infection always takes place through the stem end or wounds. Once the fungus gains entrance to the inner tissues of the fruit it develops steadily. The conclusion to be drawn from this is that the uninjured rind acts as a barrier. Since it was found that in diseased lemons the fungus was absent in the outer layers of the rind and was usually abundant in the albedo tissues, it became evident that the outer layers of the rind were in some way unsuited to growth of the fungus within them. As the oil glands are found only in the outer layers, and since the oil inhibits the fungus when added to susceptible tissue and cannot be used by the fungus even in small quantities as the only source of carbon in cultures, it seems probable that the presence of this oil is the main barrier to invasion.

It was pointed out that the fungus enters the fruit mainly through the exposed vascular strands and that the hyphae usually concentrate in the nearby parenchyma. These strands are abundant in the albedo and the core, both of which are free from the oil glands.

In the pulp the main protection against invasion by the mycelium may be attributed to the high acidity of the juice. The percentage of citric acid may be as high as 8.4 per cent (Haas, 1917) and the H-ion concentration of lemon juice extracted from thoroughly macerated pulp may range from pH 2.2 to 4.4 with an average of 2.31 for a large number of determinations from mature lemons (Bartholomew, 1923). Occasional tests of many of the media on which the fungus mats had grown showed an H-ion concentration of pH 4.7 to 5.2. Further evidence was obtained by testing the organic acids as the sole source of carbon, when it was found that growth developed only in the flasks having pH values of 7.6, 3.6, and 3.2 respectively. No growth took place at pH 2.6 and lower values. Since the lemon juice has an average pH value of 2.31 it seems probable that this high acidity protects it from the growth of the fungus, and probably also to a certain extent protects the juice-bearing tissues from invasion.

In a study of the enzymes produced by *Phomopsis californica*, cytase, pectinase, invertase, maltase, emulsin, proteases, amidase and lipase were found to be present. Pectase and inulase were tested for, but not found. Both the mycelial extract and the powdered mycelia

failed to coagulate pectin. In case of inulase, the fungus could not use the inulin as the sole source of carbon; neither the mycelial extract nor the powdered mycelia could hydrolyze inulin, as was shown by the inability of the treated media to reduce Fehling's solution. This shows that the fungus lacks both intracellular and extracellular pectase and inulase.

The growth of a fungus on a solid medium containing cellulose as the only source of carbon was indirect proof that this fungus secretes cytase which hydrolyzed the cellulose.

The ability of *Phomopsis californica* to attack cellulose was evident from its growth on pure filter papers. Microscopically, however, it was seen that the cytase in *Phomopsis californica* was limited since the cell walls were only slightly attacked. The effect upon the cell walls was much less than that produced by certain other fungi such as *Botrytis.*

With the aid of the microscope, the maceration due to the action of pectinase on the middle lamellae was seen very clearly. Even previous to the invasion by the hyphae the cells were widely separated from each other in the water-soaked advancing zone. This is shown clearly in figure 5, which was made from a core in the water-soaked area a few millimeters below the pioneering hyphae.

Since the cell walls were found to be acted upon only after they come in contact with the hyphae, and since their thickness was found to be the same in the water-soaked area and the healthy tissues, it may be concluded that the pectinase enzyme is secreted prior to and more abundantly than the cytase. This fact also may explain to some extent the pliability of the attacked rind. If cytase were very abundant the walls would be dissolved and the tissues would become soft instead of pliable.

In testing for enzymes which attack sugars it was found not only that the mycelium was able to utilize either sucrose or maltose as the only sources of carbon, but that the fungus powder vigorously hydrolyzed these sugars. Therefore, invertase and maltase were evidently present.

In testing for glucoside-splitting enzymes it was shown that emulsin was abundantly secreted. It is known that the glucoside hesperidin becomes evident in frozen citrus fruits. The widely-distributed astringent compounds known as tannins are also glucosides and may be found in very small quantities in citrus fruits. It has been shown that of various organic acids tested as the sole source of carbon, tannic acid gave the best growth. Tannins could be readily hydrolyzed, yielding some glucose for the nutrition of the fungus.

Early in this investigation it was found that the living fungus mycelium was unable to use lemon oil as a sole source of carbon, but when the mycelium was powdered to test the intracellular enzymes, it was found to have the ability to break down esters, as well as the lemon-oil emulsions, into acids (table 5). This indicates that, although the fungus appears to lack lipase as an extracellular enzyme, it contains this enzyme to some extent intracellularly. Since this enzyme remains within the living fungus cells it would not be expected to act upon the oil of the oil-bearing tissues, and this oil would therefore remain as a protection against invasion by the young advancing hyphae. It is possible that in the older diseased tissues, where the fungus cells break down the intracellular lipase may be liberated and thus be able to act upon the oil.

The general conclusion regarding the enzymatic activities of this fungus based on the foregoing experiments, may be summed up as follows:

Once the fungus gets into contact with either the albedo or the core tissues, the pectinase which it secretes enables it to dissolve the middle lamellae and cause maceration. This may be followed by secretion of cytase, enabling the fungus to get in contact with the protoplasm. Inside the cells the sugars may be used as a source of energy through the agency of carbohydrases, such as invertase and maltase. In the meantime proteases may be secreted, thus breaking down the proteins into amino acids and amides. The latter may then be acted upon by the enzyme amidase, which causes the liberation of ammonia. As the decay proceeds the young hyphae continue to grow and produce more and more enzymes. These enzymes and their by-products accumulate and diffuse ahead of the pioneering hyphae, thus paving the way for a more rapid invasion.

SUMMARY

In this paper, in which the effect of *Phomopsis californica* on the tissues of the lemon fruit was studied, there are three topics considered, namely: A comparison between the tissues of normal and diseased lemons, the relation of the oil-bearing and the juice-bearing tissues to the growth of the fungus, and the enzymatic activities causing the decay.

A method of preparing and staining the diseased tissues is given which proved to be very satisfactory. Magdala red and light green were used as differentiating stains.

In studying the anatomy of the fruit, the vascular bundles, which are the principal channels for the invasion of the fungus, were found to branch into three circles at the button (receptacle), just before entering the fruit. The first circle proceeds straight into the core. The second follows between the rind and pulp, while the third diverges towards the rind and forms a net of strands.

The oil glands, which are found only in the outer layers of the rind, appear to protect that portion against invasion. In a similar way the protection of the pulp appears to be due to the high acidity of the juice it contains. The albedo and the core, which are free from oil glands and contain the greater part of the vascular bundles, were found to be the most affected portions of the fruit.

The nature of the decay was studied by means of tests for the presence of various enzymes in the mycelium and its products. To study the effect of such enzymes on the lemon tissues, a method was developed by which certain quantities of sterile mycelial extracts could be drawn into the fruits. Only the enzymes likely to be most important were tested for. The methods commonly used in identifying such enzymes were employed.

Concerning the carbohydrases, positive evidence was obtained of the presence of cytase, pectinase, invertase, maltase, and emulsin, but negative results were obtained in the tests for pectase and inulase.

Among the other enzymes found to be very active were the proteases and amidase. The ammonia liberated by the action of the amidase on the amides was probably responsible, at least to some extent, for the effect found ahead of the pioneering mycelial threads in the fruit.

In the diseased fruit the advancing zone which lies between the margin of discoloration and the healthy tissues is mainly due to the accumulation of enzymes and their by-products.

Lipase was present in the ground mycelium and was more active on methyl and ethyl acetates than on lemon-oil emulsion.

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HEAT PENETRATION IN THE PASTEURIZING OF SYRUPS AND CONCENTRATES IN GLASS CONTAINERS

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INTRODUCTION

The enforcement of the food and drug laws during the past decade has had a marked influence on the methods used for the preservation of fruit juices and syrups. Chemical preservatives, at one time very generally used, have been to a large extent replaced by pasteurization. The only chemical preservatives permitted by law are sodium-benzoate and sulfur-dioxide. Used in moderate concentrations these preservatives are considered not injurious to health; but the consuming public has reacted unfavorably toward them in recent years, largely because they affect the flavor of the product adversely.

Since pasteurization is the method best suited to the preservation of these beverages, it is very desirable that accurate information regarding the factors affecting this operation should be available.

Pasteurization is that process of food preservation in which the food is heated to a temperature sufficient to destroy or inhibit the growth of any microorganisms that would affect the food injuriously, but not necessarily to destroy all the living microorganisms with which the food may be infected.

In the pasteurization of fruit juices, beverages, and syrups, the time and temperature of heating must be such as to destroy the yeasts and molds the activity of which would cause deterioration of the product. Although pasteurized fruit-juice products are not

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sterile, their acidity and sugar content act as mild preservatives and prevent the activity and multiplication of the bacteria present. They have for bacteria an antiseptic rather than a germicidal action.

No uniform system of pasteurizing fruit juices and syrups is at present in use. Various temperatures and various periods of time are used by different manufacturers. In some cases these are excessive and injure the products by overheating, while in others they are not sufficient and permit considerable spoiling. Standardization of the methods of pasteurization is desirable. The time of heating should be as short and the temperature as low as is compatible with the destruction of all microorganisms that might spoil the product. The time necessary for pasteurization depends on certain factors, the most important of which are:

1. Lethal rate[4] of the microorganisms which initially infect the product. This depends upon the nature of the product, especially its pH value, and on the extent and kind of infection.

2. The rate of heat penetration into the product, that is, the rate of rise of temperature at the center of the product when heated in a container.

Extensive experiments have been conducted by the United States Department of Agriculture, various can companies, and by the National Canners' Association, on heat penetration in canned foods, but little has been done on the rate of heat penetration in fruit juices and syrups in glass containers.

Purpose of the Investigation.—The purpose of these experiments was to determine the rate of heat penetration into various fruit juices, syrups, and concentrates under the conditions of pasteurization to be described, and to correlate this with the time required for the temperature in the center of different-sized bottles containing various liquids to reach the pasteurizing point. These investigations were undertaken in 1924 and were completed in May, 1927.

Factors Affecting Heat Penetration.—Magoon and Culpepper[5] conclude from their preliminary experiments "that the factors affecting the rate of change of temperature at the center of the can are the diameter of the container, the conductivity, thickness, and radia-

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[4] The term lethal rate was introduced by Bigelow, who defined it as the fraction of the microorganisms killed per minute, or the reciprocal of the number of minutes required to destroy all spores of the organism at a given temperature and under the conditions obtaining within the food. (See: Bigelow, W. D., *et al.* Heat penetration in processing canned foods. National Canners' Assoc. Research Bul. 16 L: 3–128, 89 figs. 1920.

[5] Magoon, C. A., and C. W. Culpepper. A study of the factors affecting temperature changes in the container during the canning of fruits and vegetables. U. S. Dept. Agr. Dept. Bul. 956: 1–54. 57 figs. 1921.

tive power of its walls, the temperature, conductivity, and mobility of its contents, and the temperature, conductivity, and movement of the medium surrounding it."

Bigelow[6] points out that heat penetration is influenced by the size of the container, the initial temperature of the food, the retort temperature, the nature and consistency of the food, and by the presence of forced convection currents due to the rotation of the container during processing.

Graph 1   HEAT PENETRATION IN VARIOUS FRUIT CONCENTRATES

1 - Pasteurizer
2 - Water
3 - Tokay Conc - 70° Balling
4 - Mixed Loganberry, Lemon and Orange Conc - 50° Balling
5 - Lemon Conc. - 50° Balling
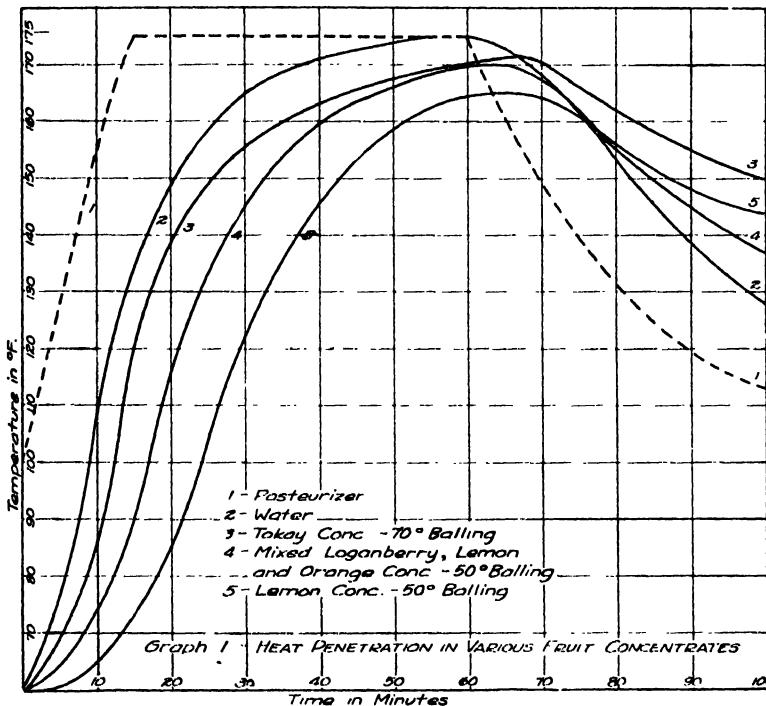
That the nature of the product is an important factor affecting heat penetration is evident from a consideration of graph 1 showing heat penetration in various fruit concentrates in gallon glass bottles.

An examination of graph 1 will show that heat penetration is most rapid in 70°-Balling Tokay-grape concentrate and slowest in the 50°-Balling lemon concentrate. A 72°-Balling orange concentrate, not shown in the graph, approached the lemon closely.

Evidently the rate of heating is not in the order of decreasing concentration but depends on other factors. Although the concentration of the product is not the only factor which determines the rate

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[6] Bigelow, W. D., *et al.* Heat penetration in processing canned foods. National Canners' Assoc. Research Bul. 16 L: 3-128. figs. 1-89. 1920.

of heat penetration into the product, it has a considerable effect, as may be seen by comparing graphs 1 and 2. A comparison of the two will show that heat penetration in general decreases with increasing concentration.
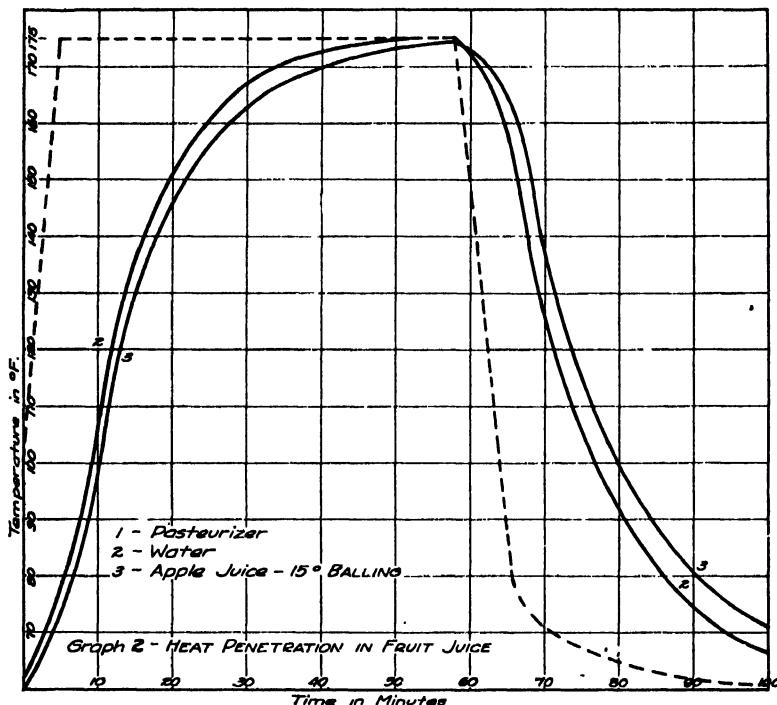
Graph 2 shows the typical heat penetration in fruit juices in gallon glass bottles. In this test heat penetration was determined in the following fruit juices:

20° Balling-muscat grape juice	15° Balling-apple juice
20° Balling-strawberry juice	11° Balling-lemon juice
18° Balling-pomegranate juice	

It was found that although the heat penetration in these juices did not differ very greatly from that in water, it was appreciably slower. Heat penetration was least rapid in the apple juice and most rapid in the grape juice, but the differences in rates in the various juices were so slight that they could not be shown in the graph. Apple juice was therefore taken for the purpose of illustration.

It is known that oranges and lemons are rich in pectin. Furthermore the concentrates of these fruits, at the time these experiments were made, appeared somewhat gelatinous. Concentrated Tokay-grape juice, on the other hand, is low in pectin and gum content. It

showed the most rapid heating, although its concentration was 20° Balling greater than that of the lemon concentrate.

This difference in the rate of heat penetration in the fruit juice concentrates is probably due to difference in viscosity occasioned by differences in the content of pectin and gums.

In the case of the juices it is known that apple juice is richer in pectin content than grape juice. This difference probably caused the difference observed in the rate of heat penetration.

Apparently, then, the rate of heat penetration will depend greatly on the viscosity of the product. This viscosity will be affected by the presence of gums and pectins and the concentration and acidity of the product.

In general then the rate of heat penetration depends upon:

1. The size and shape of the container and the thickness and conductivity of its walls.

2. The viscosity and the rate of change of viscosity with change in temperature of the product.

3. The method of heating the product.

In these investigations we confined ourselves to the study of factors 1 and 2.

EXPERIMENTAL PROCEDURE

Preparation of Materials.—

1. Simple Syrups.—Simple syrups of distilled water and cane sugar were made with Balling degrees (percentages of sugar by weight) of 10, 20, 30, 40, 50, 60 and 70.

2. Fruit Juice Concentrates and Syrups.—The following concentrates were made by concentrating the respective juices in vacuo:

> Orange concentrate—72° Balling
> Tokay-grape concentrate—70° Balling
> Mixed loganberry, lemon, and orange concentrate—50° Balling
> Lemon concentrate—50° Balling.

3. Fruit Juices.—Lemon juice of 11° Balling, strawberry juice of 20° Balling, apple cider of 15° Balling, Muscat grape juice of 20° Ball, and pomegranate juice of 18° Balling were prepared as described in Bulletin 359.[7] These were not filtered clear, although they were free from sediment.

4. Pectin Syrups.—Powdered citric acid, of a fair degree of purity, and pectin[8] were added to simple syrups of 40° and 70°

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[7] Cruess, W. V., and J. H. Irish. Fruit juice investigations. California Agr. Exp. Sta. Bul. 359: 525–568. 1923.

Balling. The citric acid dissolved readily but the pectin with diffi-
culty. The pectin was incorporated with the sugar and acid by
rapid, mechanical stirring and heat. This series included the following
solutions:

> 40° Balling simple syrup and 0.5 per cent citric acid.
> 40° Balling simple syrup and 1.5 per cent citric acid.
> 40° Balling simple syrup, 0.5 per cent citric acid and
>   1 per cent pectin.
> 40° Balling simple syrup, 1.5 per cent citric acid and
>   1 per cent pectin.
> 70° Balling simple syrup and 0.5 per cent citric acid.
> 70° Balling simple syrup and 1.5 per cent citric acid.
> 70° Balling simple syrup, 0.5 per cent citric acid and
>   1 per cent pectin.
> 70° Balling simple syrup, 1.5 per cent citric acid and
>   1 per cent pectin.

5. Simple Pectin Sols.—Solutions of 0.5, 1.0 and 1.5 per cent
pectin were prepared by allowing the required amount of pectin to
soak in a limited amount of water until dissolved and then diluting
to the proper strength. Any lumps of pectin which formed were
broken up with a spoon occasionally during soaking. No heat or
mechanical stirring was used to aid solution. In this manner sols of
the maximum viscosity resulted.

6. Acidified Pectin Sols.—To pectin sols made as in (5), enough
powdered citric acid was added to bring the acidity to 1 per cent as
citric.

7. Pectin Sugar Syrups.—To pectin sols made as in (5), enough
sugar was added to give solutions of 40 per cent and 55 per cent,
sugar respectively.

8. Acidified Pectin-Sugar Syrups.—To syrups prepared as in (7),
1 per cent of citric acid was added.

Containers.—Gallon, half-gallon, quart, pint, eight-ounce, and
four-ounce glass bottles were used (fig 1) to determine the effect of
size. These are the bottles commonly used in commercial practice.
Care was taken that bottles of the same size had the same distribution
of glass, i.e., bottles of the same height and weight.

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[8] In these experiments powdered citrus pectin was used. It was free from
sodium acetate and sugar but was slightly acid in reaction. Its analysis, fur-
nished by the Exchange Lemon Products Company, was as follows:
  Grade—165 jelly units (forms jelly with 165 parts of sugar).
  Moisture—10 per cent.
  Ash—3.5 per cent.
  Acidity towards methyl red—0.9 per cent as hydrochloric.
  Acidity towards phenolphthalein—1.7 per cent as hydrochloric.

To determine the effect of form, various types of pint glass containers were used (fig. 2): slender-necked and short-necked grape-juice bottles, milk bottle, Mason fruit jar, and tall and flat syrup jugs.

Gallon glass bottles were used in all other tests because their greater volume made more accurate measurements possible.

Fig. 1. Bottles used for simple syrup.

Fig. 2. Pint glass containers of various shapes.

Methods of Measuring Temperature.—In the preliminary tests in the Fruit Products Laboratory at Berkeley, glass thermometers were used to indicate the temperature at the center of the bottles. These were soon abandoned for thermocouples constructed of copper and constantan. The first hot junctions used were incased in metal sheaths,

but Bakelite sheaths were used later.[9] The hot junction was soldered
in a copper tip which screwed into a piece of Bakelite tubing. By
means of a one-hole rubber stopper, this was so placed in the bottle
that the tip was at approximately the center of the contents (fig. 3).
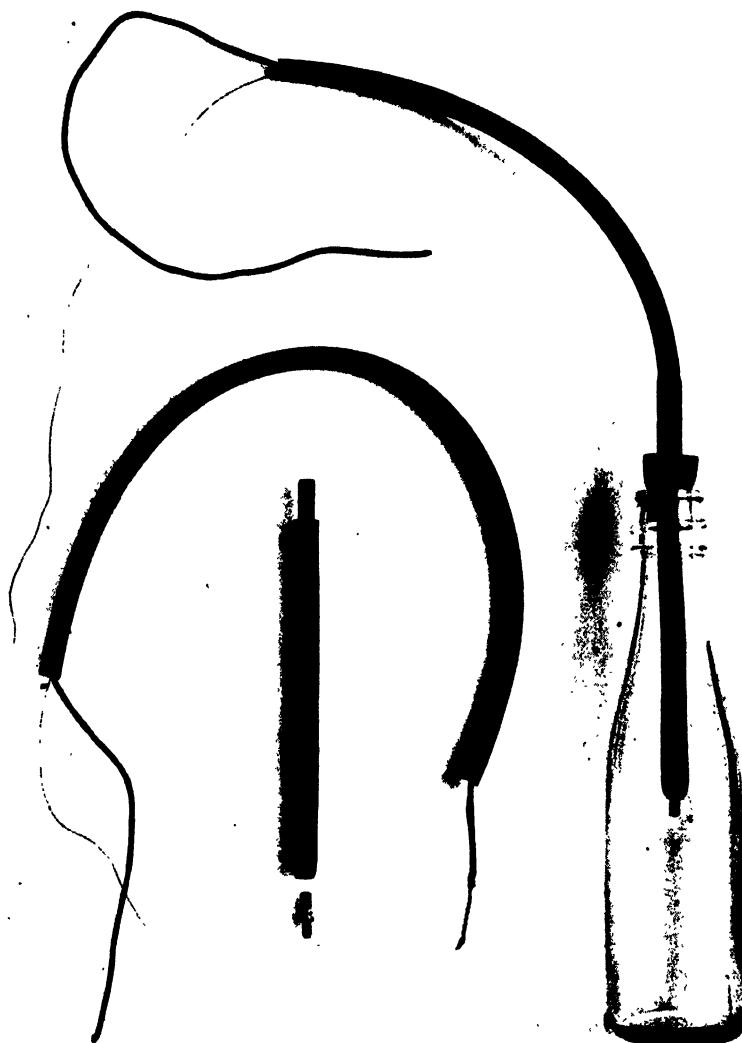
Fig. 3. Showing position of hot junction in the bottle and the parts of the
thermocouple sheathing.

The rubber stopper allowed no leaks and was wired to the neck of the bottle.

The leads from the hot junction were protected by being incased in rubber tubing and the cold junction was placed in the potentio-meter box (fig 4). A Leeds and Northrup potentiometer indicator reading directly in degrees Fahrenheit and equipped with a cold-junction compensator was used. The potentiometer was in all cases calibrated for the conditions under which measurements were made, and the thermocouples were frequently checked against standard mercury thermometers.
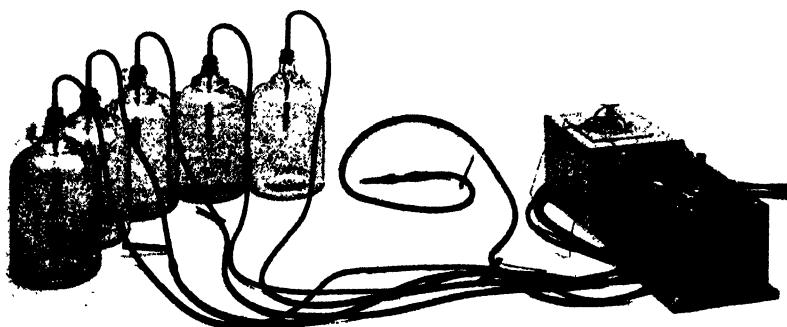
Fig. 4. Showing method of arrangement of potentiometer, junction box and thermocouples.

In order that temperature measurements could be made with the one potentiometer indicator and with more than one hot junction, a junction box[10] was used. In the final arrangement in the Fruit Products Laboratory ten thermocouples were used. This arrangement was essentially that used in the Fruit Products retort laboratory in San Francisco, where temperature measurements were made with twenty thermocouples in some of our experiments.

Method of Pasteurizing.—In preliminary tests a small pasteurizer 20 by 20 by 30 inches heated by steam jets was used for heating the bottles. It was difficult to maintain a uniform temperature because the pasteurizer was not equipped with an automatic temperature control. The water in the pasteurizer was agitated by intermittent stirring with a paddle. However, in spite of lack of automatic control, fairly consistent results were obtained. These data have not been used in this paper.

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[10] For a detailed description see: Foote, P. D., C. O. Fairchild, and T. R. Harrison. Pyrometric practise. U.S. Bur. of Standards Technologic Paper 170: 83. 1921.

The equipment used in the first set of experiments, the data from which were used in this paper, consisted of a vertical canning retort arranged for use as a discontinuous pasteurizer with automatic temperature control and an air agitator to insure uniform heating of the water bath.[11]

It has been established that ordinary yeasts are destroyed at or below 150° F, and most molds at a temperature of 175° F. In these investigations, 175° F was taken as the pasteurizing temperature. In all cases the temperature of the water was raised to 100° F, before submerging the bottles, which were at room temperature.

The thermocouples were placed in the bottles with the points as nearly as possible at the center of the contents of the bottles. The bottles were firmly fixed in an iron basket to prevent deviation of the thermocouple point, and the basket was suspended midway in the pasteurizer. The temperature control was set at 175° F, and the time and temperature recorded at five-minute intervals.

With all of the simple syrups the water in the pasteurizer reached 175° F within ten minutes, while with the fruit-juice concentrates it required fifteen minutes. This difference in the rate of heating was due to fluctuation of pressure in the steam line.

The pasteurizer in the Fruit Products Laboratory in Berkeley was used in the second set of final experiments. During these experiments, the water in the bath was continuously agitated by compressed air. The heating was controlled very carefully by hand. The maximum variation in the temperature of the bath was 2° F.

As in experiments carried out in the retort laboratory in San Francisco, the bath was raised to 100° F before submerging the bottles, which were at room temperature when placed in the bath. Then the temperature was rapidly raised to 175° F. In all experiments this temperature was attained within five minutes, as high-pressure steam was used. By means of hand control, the bath temperature in the first few experiments was maintained to within 2° F of 175° F. In later runs the range was within 1° F.

In filling the bottles, care was taken to allow for expansion, as otherwise the bottles would burst during heating. The bottles were placed in a specially devised wire rack which prevented motion of the bottles during the heating and subsequent cooling and helped to keep the hot junction at the exact center of the bottle.

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[11] A description of the retort will be found in the catalog of the Sprague Sells Corp., of Chicago; of the potentiometer temperature-measuring equipment in the catalog of the Leeds & Northrup Company; and of the temperature controller in the catalog of the Foxboro Instrument Company.

Methods of Cooling.—For the cooling period, the steam was turned off, and cold tap water was allowed to enter at the bottom of the retort and displace the hot water, which escaped through an overflow valve at the top. The water in the bath was agitated by means of compressed air during this cooling as well as during heating. The cooling was fairly uniform in all experiments.

During the heating and cooling periods, temperature readings were taken frequently, usually at intervals of 2 minutes.

Accuracy of the Experimental Work.—The data obtained in these investigations were determined with a degree of accuracy sufficient for the purpose in hand, which was to introduce certain improvements into the practice of pasteurization. From the graphs, the time for pasteurization may be determined to within five minutes. The potentiometer was accurate to within 1° F.

THE THEORY OF HEAT PENETRATION

Before considering the results of the experiments, it may be well to consider briefly the more important aspects of the theory of heat penetration.

Heat may flow from a warmer body to a colder body or from one portion to another of the same body by any one or all of three ways, conduction, convection, and radiation. As the conductivity of most liquids is low, the rate at which heat is transferred in such media by simple conduction is slow. The flow of heat through any medium or through vacuum by radiation is proportional to the fourth power of the temperature drop. In the process of pasteurization, the heat transferred by radiation is usually negligible. The rate at which heat is transferred by convection is proportional to the temperature difference, in accordance with Newton's law. Where the viscosity of the medium is such as not to interfere with or impede convection currents, the rate at which heat is transferred by this process in liquids is rapid.

When the bottle is placed in the pasteurizer, heat enters from all sides and the coldest portion is, theoretically at least, always at the center. The surface of the contents will rapidly reach the pasteurizing temperature, but the time required will vary according to the conductivity and the thickness of the glass walls of the container. If the viscosity of the fluid is such as to impede convection currents, the rate of heat penetration into the interior will be slow. Where the viscosity is low enough to freely permit convection currents, the

temperature in the center will rapidly reach the pasteurizing temperature, although the time required is also dependent upon the size and shape of the containers.

The influence of viscosity will be further discussed in considering the results of the experiments.

If all other conditions are kept constant, the maximum rate of heat penetration will occur as pointed out by Bigelow[12] in those products which contain a minimum amount of substances in solution and in which the liquid is of such a nature that it affords the maximum freedom for movement of convection currents. In such a product heat penetration is due chiefly to convection, but conduction also exerts its full force. In a product where the viscosity or consistency is such as to inhibit or eliminate all convection currents, we get the minimum rate of heat penetration.

The rate of heat penetration in fruit juices which do not contain gums and pectins in solution will approach the maximum. Heat penetration in jellies and in concentrated syrups rich in gums and pectins will approach the minimum.

RESULTS OF EXPERIMENTS

The temperature readings taken during heating and cooling of the containers in the various experiments have been plotted against time in graphs 1 to 10. The data tables have been omitted in order to conserve space; the graphs illustrate the results much better than do tables.

The graphs show only the general form of the heat-penetration curves under certain typical conditions. They do not represent all the data taken. Deviations from these graphs are discussed in the text where the specific experiments are considered.

INFLUENCE OF SUGAR AND OF SIZE AND SHAPE OF CONTAINER

In view of the fact that syrups are being marketed to a very large extent in glass bottles and the production of fruit syrups for beverage purposes is increasing, it was deemed advisable to obtain data on the rate of heat penetration in simple cane-sugar syrups of various densities. Information of this sort for glass containers has been lacking, and sterilizing practices among bottlers vary widely.

Cane-sugar syrups of 10, 20, 30, 40, 50, 60, and 70 per cent were placed in gallon, half-gallon, quart, pint, eight-ounce (soda pop) and

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[12] Bigelow, W. D., *et al. Loc. cit.*

Graph 3 - HEAT PENETRATION IN CANE SUGAR SYRUPS

1. Pasteurizer
2. Water
3. Syrup – 50° Balling
4. Syrup – 60° Balling
5. Syrup – 70° Balling

Graph 4 - EFFECT OF SIZE OF BOTTLE ON HEAT PENETRATION IN 70° BALLING SYRUP

1- Pasteurizer
2- 4-oz. Bottle
3- Quart Bottle
4- Half-gallon Bottle
5- Gallon Bottle
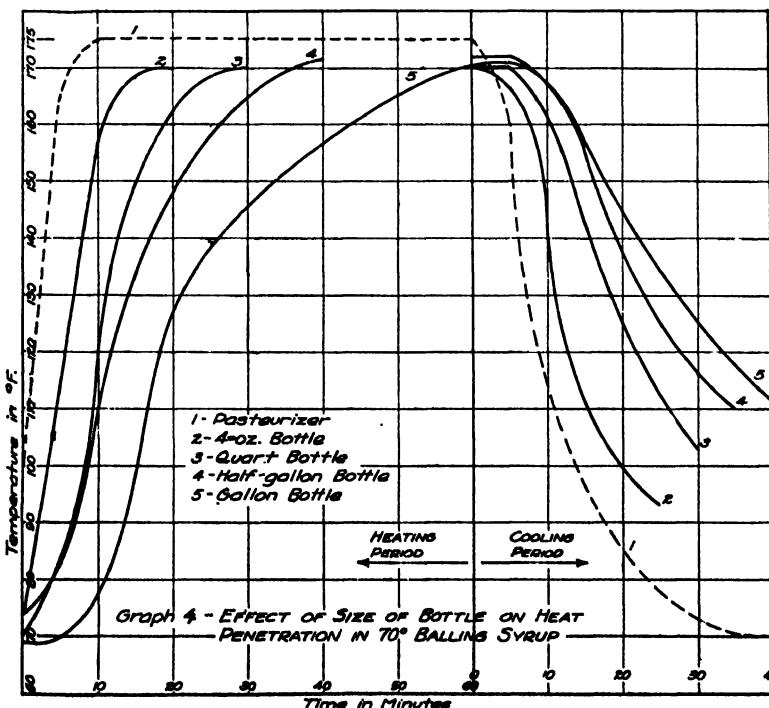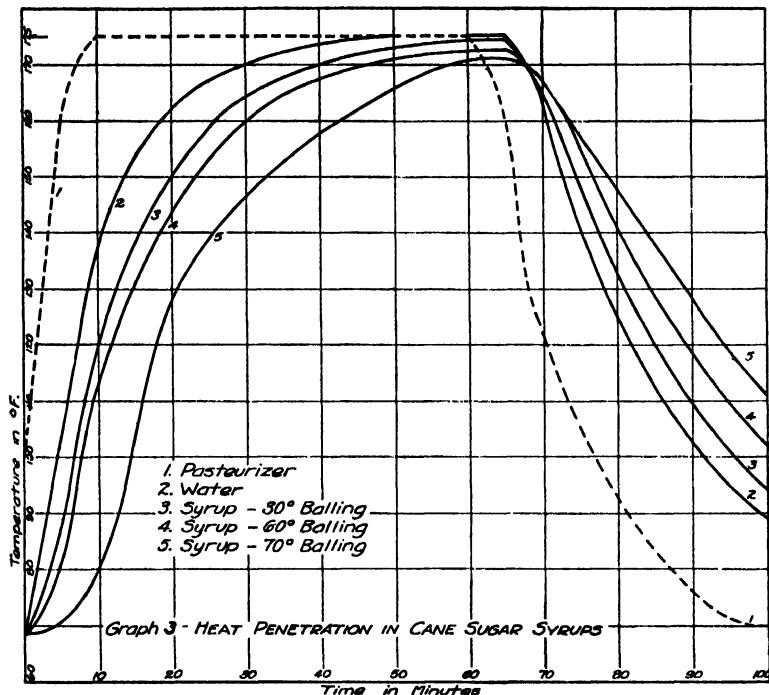
four-ounce bottles, fitted with thermocouples and heated in the pasteurizer previously described.

It was found that in the containers of each size used the rate of heat penetration decreased as the concentration of syrup increased. Graph 3, showing the heat penetration in cane-sugar syrups in gallon bottles, is typical of the results obtained, with the exception to be noted later.

It is also obvious from graph 4 that the rate of heat penetration increased with decrease in size of container.

The rates of heat penetration closely approach one another in syrups from 10° Balling to 60° Balling, but were nevertheless in the inverse order of the concentrations of the syrups. Between 60° Balling and 70° Balling, however, there was a very pronounced increase in the lag and this lag was more marked during cooling. In fact in all·cases during cooling, there is more separation of the various curves than during heating. This lag between the 70° Balling and the other syrups was not so great in the eight-ounce and four-ounce bottles as in the larger containers (see graph 5), owing to the fact that heat transfer is much more rapid in the smaller containers than in the larger. The heat transfer by conduction is more rapid, because in the smaller containers there is a greater heating surface per unit volume, and the heat wave has a shorter distance to travel to reach the center. Thus the temperature of the contents increases, and hence the viscosity decreases, thus allowing rapid convection even in the case of the more concentrated syrups.

If we keep the size of the container constant and change its shape, the heating surface per unit volume will change. The more nearly a container approaches a sphere in shape, the less is its heating surface per unit volume, and the less is the rate of heat penetration in that container, assuming all other factors to remain constant.

There is on the market a great variety of glass containers. Each fruit-products manufacturer desires an individual bottle for his product. Moreover, the types of containers vary with the product. Nevertheless, there is a certain degree of standardization. Familiar forms of glass containers are those used for milk, the Mason jar for home preserves, syrup jugs, and bottles used for cider and grape juice.

To determine whether the effect of shape was marked, investigation was made of heat penetration in water and in 68°-Balling simple syrup in the following pint-size glass containers: slender-necked and short-necked bottles, milk bottle, Mason fruit jar, and tall and flat syrup jugs (see fig. 2). In each of these was placed exactly 400 cc of the fluid investigated. The copper tip of the hot junction was

fixed at approximately the center of the contents.   They were then pasteurized as previously described.

The experiments showed that in the various containers studied there was no very marked difference in heat penetration with either water or syrup, probably because there was no large variation in the heating surface per unit volume in these containers.   Moreover the glass in the bottles which had the greatest heating surface was much thicker than in the Mason jar, which had the smallest.

Graph 5 - RELATION OF SYRUP CONCENTRATION AND SIZE OF BOTTLE TO HEAT PENETRATION
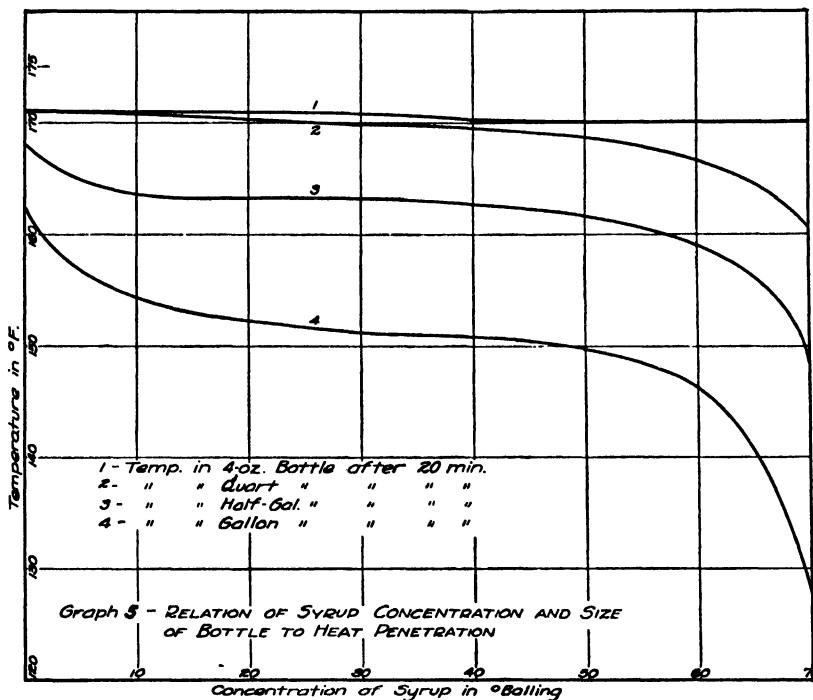
The thickness of the glass walls of the container is an important factor and the effect of increasing this thickness is to decrease the rate of heat penetration.

From graphs 3, 4, and 5, we may conclude that sugar in dilute water solutions has very little retarding effect on the heat penetration. However, when the concentration is high, the effect on heat penetration is very appreciable, and the rate of heat penetration decreases as the concentration of the solution increases.   This decrease in the rate of heat penetration is caused by an increase of viscosity, due to the dissolved sugar, which has an impeding effect on convection currents.   The sugar in dilute solutions does not greatly increase the viscosity, but in concentrated solutions, 60–70° Balling, it does so greatly, and thereby proportionately decreases heat penetration.

These results are in accordance with theory and in agreement with the work of other investigators. The effect of viscosity of sugar solutions is discussed fully below.

Bigelow[13] has found that sugar syrups of 10, 20, 30 and 50 per cent in tin cans diminish the rate of heat penetration in the order named.

Magoon and Culpepper[14] have found that sugar solutions show no marked effect upon the rate of changes of temperature at the center of tin cans where the concentration was 10 per cent or less. Even in a 60-per-cent solution the effect was not very marked. Their very interesting and important discussion of the effect of sugar on heat penetration follows:

"The effect of the sugar solution upon the rate of change of temperature at the center of the can is due to the greater viscosity, which decreases the rate of convection in the sugar solutions. The value of the force which tends to produce convection currents in the solution depends upon the steepness of the gradient between the temperature at the center of the can and the temperature at the margin of the solution, so that the force tending to produce convection currents becomes less and less as the temperature at the center of the can approaches that of the bath. It is known that the viscosity of sugar solutions decreases as the temperature increases. It is this characteristic of sugar solutions that makes the temperature shown by the upward curves follow so closely that of distilled water.

In the cooling off, however, there is an increase in the viscosity. Also as the temperature at the center of the can falls, the temperature gradient between the center and the margin becomes flatter; hence, the force tending to cause convection becomes smaller, until finally the resistance due to viscosity is great enough to stop all convection, and the process becomes one of pure conduction, which is very much slower than convection. The difference in viscosity at high and low temperatures makes the cooling curve much different from the upper curve."

The cause of the abrupt lag of the curve at 70° Balling must then be the high viscosity of syrup of this concentration. This viscosity is evidently high enough to markedly impede convection. To test this assumption, the viscosity of the syrups was determined, with the results given in table 1.

It is evident that there is a marked difference between the 70-percent syrup and the syrups of lower concentration.

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[13] Bigelow, W. D., *et al. Loc. cit.*

[14] Magoon, C. A., and C. W. Culpepper. *Loc. cit.*

TABLE 1

RELATIVE VISCOSITY OF SIMPLE SYRUPS AT ROOM TEMPERATURE

Concentration in percentage of sugar	Specific gravity	Relative viscosity*
0 (water)	1.00	1.00
10	1.04	1.05
20	1.08	1.15
30	1.13	1.43
40	1.18	2.00
50	1.23	3.10
60	1.29	6.33
70	1.35	49.00

*All viscosity measurements were made by means of a Stormer viscosimeter. They are intended to show the relative trend of the viscosity and not of its absolute value.

The relative viscosity of sugar solutions increases with increase in concentration. This increase is very slight at first, but accelerates until between 60° and 70° it is very marked. The heat penetration that could be predicted from a consideration of these results is in accord with that actually found. Evidently viscosity is an extremely important factor in heat penetration.

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INFLUENCE OF PECTIN

From what has been said concerning the simple syrups, it is seen that in solutions of crystalloids such as sugar, the viscosity does not markedly differ from that of water until high concentrations are reached. On the other hand, it is known that the presence of reversible colloids, such as starch and gum arabic, in water markedly affect the viscosity even in dilute solutions.

Thus Magoon and Culpepper[15] and Bigelow[16] have shown that, in starch solutions, the formation of convection currents was greatly impeded and heat penetration delayed. This was noticeable in the case of 1-per-cent and 2-per-cent solutions but became more marked as the amount of starch increased. Bigelow proved that in a starch solution of 6 per cent, heat penetration was due almost entirely to conduction.

Hence it would be expected that the presence of pectin, another reversible colloid, would markedly affect the rate of heat penetration.

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[15] Magoon, C. A., and C. W. Culpepper. *Loc. cit.*
[16] Bigelow, W. D., *et al. Loc. cit.*

To show conclusively that this is the case, the following series of experiments was made.

Simple Pectin Sols.—Pectin sols with concentrations of 0.5, 1.0, and 1.5 per cent were prepared as previously described. These were pasteurized in gallon bottles.

The results of the experiments showed that pectin in solution retarded heat penetration. Curve 4 in graph 9 is typical of the behavior of pectin sols. The retardation was greater in the more concentrated solutions.

Acidified Simple Pectin Sols.—Pectin sols of the above concentrations with 1 per cent of citric acid added were pasteurized in gallon bottles. Although here too the retardation of heat penetration was appreciable (see curve 3 in graph 9), it was noticeably less than in the former experiment.

The addition of acid probably favored finer dispersion or aided the hydrolysis of pectin to insoluble pectic acid, which would be precipitated from solution. That it had an effect on the relative viscosity may be seen from table 2.

TABLE 2

RELATIVE VISCOSITY OF PECTIN SOLS AT ROOM TEMPERATURE

Per cent of citric acid added	0.0 per cent pectin	0.5 per cent pectin	1.0 per cent pectin	1.5 per cent pectin
			Relative viscosity	
0	1.00	1.13	1.48	2.08
1	....	1.00	1.04	1.93

Cane-Sugar Syrups Containing Pectin with and without Added Acid.

Four lots each of simple syrup of 40° Balling and 70° Balling were made. The first contained 0.5 per cent and the second 1.5 per cent citric acid. The third contained 0.5 per cent citric acid and 1.0 per cent pectin; the fourth contained 1.5 per cent citric acid and 1.0 per cent pectin. These were pasteurized in gallon and pint bottles.

It was found that the presence of pectin had a decided retarding effect on the rate of heat penetration. Graph 6 is typical of the results obtained.

However, the presence of acid in simple syrups had a decided tendency to hasten the rate of heat penetration, especially as the concentration of acid was increased. This was perhaps due to inversion of the cane sugar by high concentrations of acid. The presence of acid in pectin sugar sols also had some tendency to hasten the rate

of heat penetration, probably owing to reduction in viscosity of pectin due to increased acidity.

The syrups of the above series to which pectin was added contained also acid; and in preparing them relatively high temperature and vigorous mechanical stirring were used. This probably accounted for the fact that they did not jell, a result contrary to the findings of Lal Singh[17] and others. Wendelmuth[18] has found that pectin solu-

1 - Pasteurizer
2 - Water
3 - 70% Sugar, 1½% Citric Acid
4 - 70% Sugar, 1½% Citric Acid
   1% Pectin (not jellied)

Graph 6 - EFFECT OF PECTIN ON HEAT PENETRATION IN ACIDIFIED 70° BALLING SYRUP

Time in Minutes
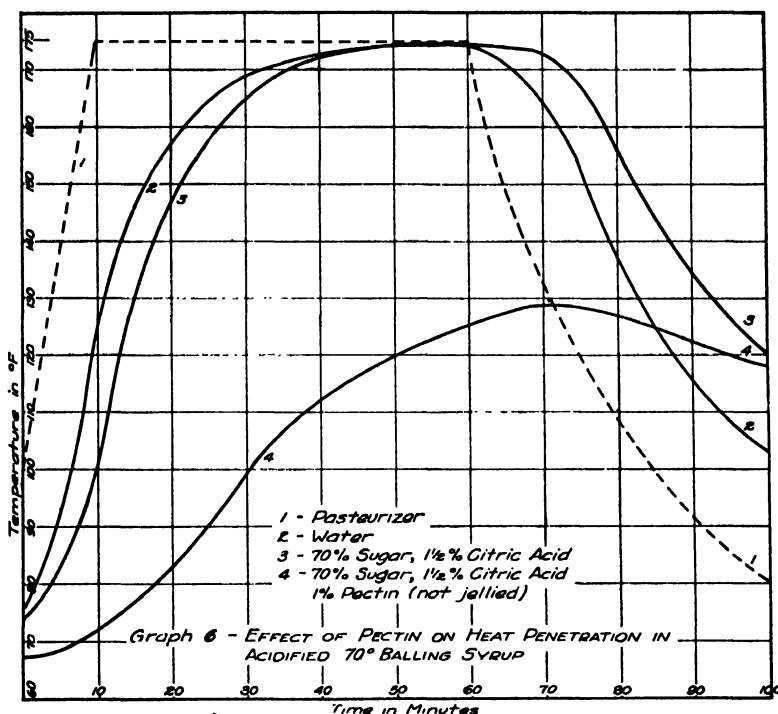
tions which were excessively stirred or heated did not jell, even though the proper amount of acid and sugar was present.

In pectin sols, the results obtained depend upon the manner of preparation. Heating and rapid mechanical stirring, especially in the presence of acid, tend to reduce the viscosity of the pectin sols by hydrolysis of the pectin to a less highly methylated state and by the coagulation of particles in suspension. Experiments have shown

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[17] Singh, Lal. Important discoveries in jelly making. Canning Age 3:5–8. 1922.

Also, Practical experiments in jelly making. Canning Age 3:11–14. 1922.

[18] Wendelmuth, Gerta. Über die Gelierfähigkeit von Obstsäften und Pektin-lösung. [On the jellying ability of fruit juices and pectin solutions.] Kolloid-chemische Beihafte 19: 115–137. 1924.

that the difference noted in graph 7 is due rather to the use of heat in preparing the products than to a difference in acid content, although the difference in acidity also contributes to the effect noted.

In our second experiment with pectin, 0.5 per cent, 1.0 per cent, and 1.5 per cent of pectin were added to 40° Balling and 55° Balling simple syrups. To one series 1 per cent of citric acid was also added. These materials were incorporated in the manner previously described to yield syrups of maximum viscosity. They were pasteurized in gallon bottles.

1 - Pasteurizer
2 - Water
3 - 40% Sugar, 1% Citric Acid
4 - 40% Sugar, 1% Pectin, 1½% Citric Acid - Prepared by Heating and Stirring
5 - 40% Sugar, 1% Pectin, 1% Citric Acid - not Heated

Graph 7 - Effect on Heat Penetration of Manner of Preparing Pectin Sols
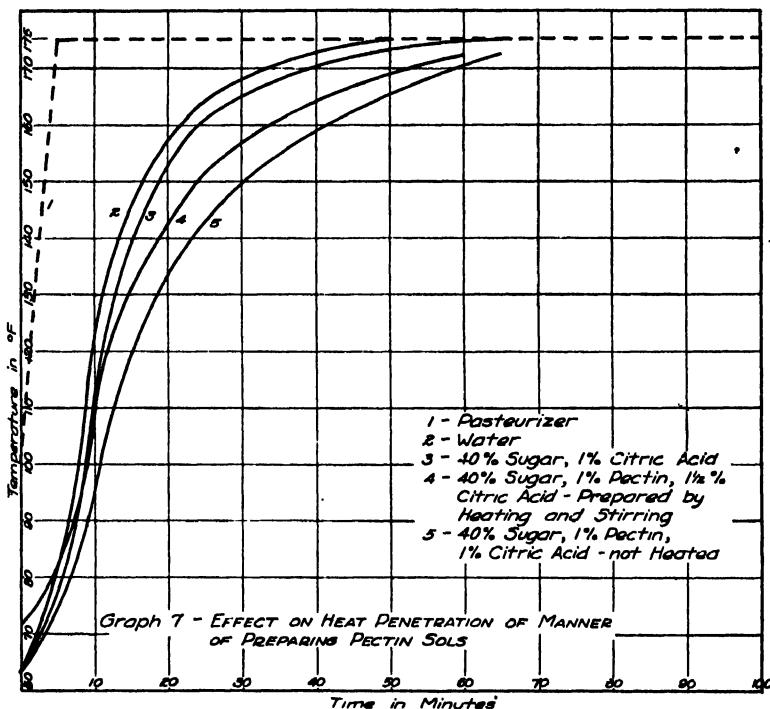
The results obtained showed that pectin markedly retarded heat penetration in syrups. This retardation increased with increase in concentration of pectin (graph 8). Acid, where it did not cause jellying, reduced this retarding effect, probably because it reduced the vicosity of pectin sol (graph 9).

This is in accordance with the work of Ohn,[19] who found that the relative viscosity of sols made from pectin, citric acid, cane sugar, and distilled water depended upon the proportion of pectin, acid and sugar present. Within certain limits, if the hydrogen-ion concen-

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[19] Ohn, Asta. Viscosity of pectin sols. J. Ind. Eng. Chem. 18: 1295–1297. 1926.

1 – *Pasteurizer*
2 – *Water*
3 – *40% Sugar*
4 – *40% Sugar – 0.5% Pectin*
5 – *40% Sugar – 1.0% Pectin*
6 – *40% Sugar – 1.5% Pectin*

Graph 8 – Effect of Pectin Concentration on Heat Penetration in Syrups

1 – *Pasteurizer*
2 – *Water.*
3 – *1% Pectin – 1% Citric Acid*
4 – *1% Pectin*
5 – *55% Sugar – 1% Citric Acid*
6 – *55% Sugar*
7 – *55% Sugar – 1% Pectin*
8 – *55% Sugar – 1% Pectin – 1% Citric Acid (jelled)*

Graph 9 – Effect of Acid on Heat Penetration in Pectin Sugar Sols

tration is sufficient, any increase in the amount of pectin or sugar added caused a noticeable increase in the relative viscosity. If the hydrogen-ion concentration is low, such increases cause only a slight change in the relative viscosity.

In this series of experiments, the retardation in the rate of heat penetration due to the presence of pectin was greater than in the former series (graph 7) for the reason stated above, i.e., that the pectin in this second series was dissolved without heating in order to secure maximum viscosity.

The consistency of a jelly is such as to prevent convection currents. Heat penetration, being caused only by conduction, is very slow (graph 9). When the solution jellied, the heat penetration was not influenced markedly by change in pectin and in acid content. Also, it was found that changing the sugar content from 55 per cent to 70 per cent did not noticeably affect the heat penetration in a jelly.

That the foregoing results could be predicted from a consideration of the viscosity of the solutions is evident from the following table. Unfortunately sugar syrups of 55° and 70° Balling containing pectin were too stiff at room temperature to permit determination of viscosity by the instrument used.

TABLE 7

RELATIVE VISCOSITY OF PECTIN-SYRUPS AT ROOM TEMPERATURE

Per cent pectin	Per cent sugar	No citric acid added	One per cent citric acid added
		Relative viscosity	
0 (water)	0	1.00	....
0	40	2.00	1.67
0.5	40	3.20	2.61
1.0	40	7.50	6.45
1.5	40	9.25	8.95

COORDINATION OF MOST IMPORTANT FACTORS

Graph 10 makes it possible to compare readily the pasteurizing time for various syrups in various containers. There are two series of curves in this graph. In one, the time necessary for the temperature of the syrup to reach 170° F in various bottles under the pasteurizing conditions previously described is plotted as abscissas against the concentration of sugar as ordinates. In the other the relative viscosity of the syrup as abscissas is plotted against the concentration of sugar as ordinates.

The use of these curves will be seen by applying them to the following examples.

Example 1.   Suppose one desires to pasteurize 40° Balling simple syrup in gallon bottles and wishes to know the pasteurizing time necessary.   By consulting the curve for the gallon bottles, it will be seen that it requires approximately 45 minutes for the temperature at the center of the container to reach 170° F.   If 170° F is the

Graph 10 – RELATION OF VISCOSITY TO PASTEURIZING TIME AT 170°F.

Relative Viscosity at Room Temperature

Concentration of Syrup in °Balling

Time in Minutes

1 – Relative Viscosity at Room Temperature
2 – Pasteurizing Time in 4-oz Bottles
3 – Pasteurizing Time in Quart Bottles
4 – Pasteurizing Time in Half-Gallon Bottles
5 – Pasteurizing Time in Gallon Bottles
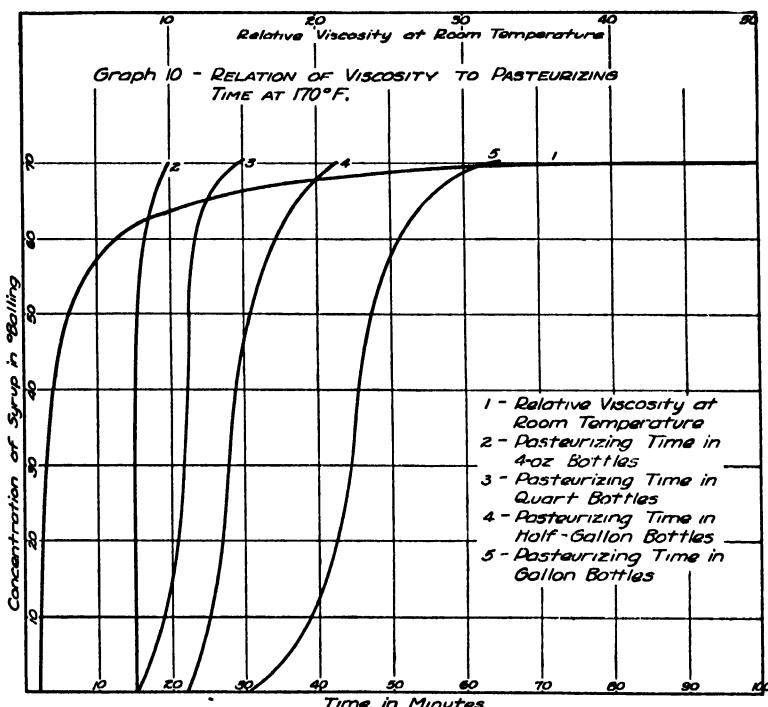
pasteurizing temperature this will be the approximate time required for pasteurization.

Example 2.   Suppose it is desired to pasteurize a fruit syrup of 40° Balling in gallon bottles and it is suspected that the viscosity of this syrup is greater than that of 40 per cent simple syrup.   Let us assume that it is found that its viscosity is roughly five times that of water.   From the viscosity curve, it is seen that the concentration of simple syrups having this viscosity is approximately 60 per cent. The time required for the temperature at the center of a gallon bottle of syrup of 60° Balling to reach 170° F is then found (as in example 1) to be approximately 55 minutes.   If 170° is the pasteurizing temperature, this will be the approximate pasteurizing time.

However, the reader must be cautioned against splitting hairs in using this graph. It will give the pasteurizing time only to within five minutes. Other factors than viscosity enter the case also; such, for example, as thickness of the walls of the container; agitation of the water in the pasteurizer and the shape of the container. The graph is presented principally to illustrate the very great effect of viscosity on heat penetration in liquids.

SUMMARY

1. The rates of heat penetration in bottled syrups as affected by sugar concentrations, viscosity of liquids, size of bottle, and variety of syrup were determined.

2. It was found that sugar exerted only a slight retarding effect at low concentrations but an appreciable retarding effect at concentrations above 50° Balling. A very marked decrease in rate occurred between 60° and 70° Balling. This was proved to be due to increase in viscosity of the solutions with increase in sugar concentration.

3. Differences in the rate of heat penetration in containers of different sizes were not very great in small containers such as pint, 8-ounce and 4-ounce bottles, but a marked difference was found in the rate between the gallon and the smaller containers. This last fact probably accounts for the greater loss by molding of fruit juice in gallon containers than in small containers; most manufacturers give little or no greater time to gallon-size than to smaller containers.

4. Syrups rich in pectins and gums transmitted heat very much more slowly than those poor in these constituents. This was proved to be caused by the effect of pectin on the viscosity. Pectin very greatly increased the viscosity of the sugar solutions of fruit juices and thereby greatly reduced transmission of heat by convection.

5. Where the juice, syrup, or concentrate actually jellied, heat penetration became very slow, but was not noticeably affected by increase or decrease of the sugar content, provided the sugar content was not reduced below that required for jelly formation.

ACKNOWLEDGMENT

FUMIGATION WITH CALCIUM CYANIDE DUST[1]

H. J. QUAYLE[2]

INTRODUCTION

When hydrocyanic acid was first used for the fumigation of citrus trees in California in 1886,[1, 2, 3] potassium cyanide, KCN, was the material used. Potassium cyanide continued to be used until 1909, when it was replaced by sodium cyanide, NaCN. Sodium cyanide had been used for industrial purposes, but probably because of the sodium chloride usually present and the consequent decomposition of hydrocyanic acid gas, it was slow in coming into use for plant fumigation. Sodium cyanide was first suggested by Lounsbury[4] for plant fumigation; the effect of the presence of NaCl on the evolution of the gas was indicated by Newell,[5] and Woglum[6] demonstrated its practical use for citrus fumigation when free from the impurity NaCl. In both of these cyanide salts hydrocyanic acid gas is evolved very slowly by simple contact with the atmosphere, so that it is necessary to add sulfuric acid and water to secure a sufficiently rapid generation.

METHODS OF FUMIGATION

The first experiments in hydrocyanic acid fumigation for citrus trees involved the use of a generator outside of the tent. This apparatus soon gave way to earthenware pots which were placed under each tree, and this method of generation was followed without change for twenty-seven years. In 1913 William Dingle designed a portable

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[1] Paper No. 171, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Professor of Entomology in the Citrus Experiment Station and Graduate School of Tropical Agriculture and Entomologist in the Experiment Station.

generator which used a solution of sodium cyanide. The addition of acid to the solution of cyanide resulted in instantaneous generation of the gas, which was conducted by its own pressure through a hose from the generator to the tent. Portable machines (drawn by a horse) were in successful use from 1913 to 1918. In 1916 Dingle demonstrated the practical use of hydrocyanic acid in liquid form for citrus fumigation, and this method is very largely used in California at the present time.

"A" CALCIUM CYANIDE[3]

Another form of cyanide in which cyanogen is combined chiefly with calcium became available about 1916. This compound, $Ca(CN)_2$, has served as the source from which liquid hydrocyanic acid has been manufactured by one of the plants built for the purpose in southern California. "A" calcium cyanide is formed by fusing calcium cyanamid, $CaCN_2$, with sodium chloride. In crude form it occurs as thin gray flakes. It contains from 27 to 30 per cent HCN. The chief impurities are sodium chloride and calcium carbide.

Use in Powdered Form.—In June, 1922, it was learned that crude calcium cyanide in flake form when placed in the burrow of rodents gave off hydrocyanic-acid gas with sufficient rapidity to kill them; it thus occurred to the writer that if these flakes were ground into a fine powder the gas might be given off rapidly enough to be applicable to citrus fumigation,[7] provided, furthermore, that the residue—since the material would have to be blown onto the tree—would be harmless. Consequently the manufacturers were asked to have prepared a small quantity of the crude flakes in powdered form. The material was first tried by dusting a few small citrus trees without covers, to determine the effect on the foliage. Later the powdered cyanide was blown under tented citrus trees by means of an ordinary hand-dusting machine. The results of the first tests were satisfactory, both in kill of scale insects and in lack of injury to the tree. Thirty orange trees were thus fumigated in August, 1922.[8] The next series of experiments, carried on in October, when a rain immediately followed, resulted in considerable injury to lemon trees. Experiments which followed during the next three or four months indicated that

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[3] Two different forms of calcium cyanide will be discussed in this paper. One will be designated as "A" calcium cyanide and the other as "C" calcium cyanide. "C" calcium cyanide, unless otherwise indicated, will be understood to contain 30 per cent HCN. Another grade of this cyanide containing 50 per cent HCN will be included in some of the experiments and this will be designated as "C" calcium cyanide (50 per cent).

atmosopheric moisture was the limiting factor in the use of this material for fumigating citrus trees, and more particularly lemon trees, in California.

Experiments in Australia.—In March, 1923, the writer had the privilege of continuing experiments on this method of fumigation in Australia.[9] Between 200 and 300 trees on six different farms were successfully fumigated in these experiments when the method was adopted commercially. The commercial fumigation continued successfully until the winter rains began early in May, when injury to the trees again occurred as it had in California.

Experimental work at Leeton, New South Wales, was carried on with a temperature range of from 55 degrees to 80 degrees F and a relative humidity range of from 37 per cent to 55 per cent. The climate in this area is similar to that of Tulare County, California, where, so far as the few tests have shown, fumigation by the same method may be carried on with safety to the tree. The dust method has practically replaced the older methods of fumigation in Australia, where about 300,000 citrus trees were so fumigated last year. The method has been extended also into areas near the coast having a higher humidity, such as at Lismore,[10] and Lisarow and Gosford.[11, 12]

Injury to Citrus Trees.—Lemon trees were more seriously injured than orange trees. It is well known that the lemon tree, *Citrus limonia* Osbeck, is more resistant to hydrocyanic acid gas than the common or sweet orange, *Citrus sinensis* Osbeck, the sour or Seville orange, *Citrus aurantium* Linn., the mandarin group, *Citrus nobilis* Lour., and the pummelo, *Citrus maxima* (Burm.) Merrill. It is a curious fact, however, that in the case of fumigation with the form of calcium cyanide under discussion, the lemon is much more susceptible than the other species of *Citrus*. It would seem logical to conclude that this difference must be due to the residue of dust left on the tree or to some product aside from HCN given off from the residue. The definite cause of this greater injury to the lemon is not as yet fully ascertained. Injury is most likely to occur when there is considerable atmospheric moisture, roughly, above 60 or 70 per cent relative humidity.

The injury is also different in nature from that of ordinary HCN fumigation, particularly as regards the fruit. Instead of definite pits occurring on the fruit as with HCN gas, when the dust is applied the entire upper surface of the lemon may be uniformly burned. Pitting, in ordinary fumigation, may occur on the under as well as

the upper side of the fruit. The difference in location further supports the belief that the dust residue is responsible for the injury. If it were an excess of HCN gas given off in immediate contact with the dust, then the orange should show more injury than the lemon, as it does in other methods of fumigation.

In addition to the fruit injury, a heavy leaf drop may also occur. The same type of injury may occur with the orange but to a less marked extent.

Recent reports from Australia indicate that injury to the lemon is the chief drawback to the dust method of fumigation when the weather conditions are not favorable. In Australia, however, the lemon constitutes but a small fraction of the citrus acreage.

Reasons for Investigating the Possibilities of Dust Fumigation.— The original purpose of simply grinding crude calcium cyanide into a fine powder and blowing it under a tented citrus tree was to obviate the necessity of having this material go through an expensive process in order to produce liquid HCN. If such material proved satisfactory it should reduce the cost of fumigation. The manufacture of liquid HCN requires a plant of considerable magnitude, and the parts in contact with the chemicals must be replaced practically every year. In addition to the cost, the question of safety to the operators is involved. Liquid HCN is a very dangerous material to handle, and all of the fatalities which have resulted in connection with citrus fumigation have occurred since the adoption of the liquid method. The liquid cannot be transported in the usual containers except by truck and it cannot be stored for any great length of time. Special precautions, such as icing, must be taken to keep the material cool in hot weather. These disadvantages would be avoided if dust fumigation should prove to be satisfactory.

Present Uses of Cyanide-Dust Fumigation.—In addition to citrus fumigation, cyanide-dust fumigation is now extensively practiced against rabbits[13, 14, 20] in Australia, for the fumigation of greenhouses,[15, 16] for dwellings,[17] storehouses, and railroad cars, and for the dusting of plants in the open (without covers) for several insect pests.

"C" CALCIUM CYANIDE

In these tests dust fumigation of citrus trees was of chief interest. Further work with the material thus far discussed was, therefore, abandoned in 1924 because there was too much danger· of injuring citrus trees under the climatic conditions prevailing in southern California.

The next procedure was to try to incorporate pure liquid HCN into powdered material that would be inert so far as the tree was concerned. Such materials as hydrated lime, calcium carbonate, talc, diatomaceous earth, kaolin, sulfur, and several others, were tried, but given up because of the decomposition of HCN or the increased cost as compared with the liquid HCN alone, which was already in satisfactory use.

Early in the fall of 1925 a small quantity of another form of dry calcium cyanide was delivered to the writer for determination of its possibilities for citrus fumigation. Tests were first made to determine its effect on citrus foliage. It was blown onto citrus trees enclosed in a tent; some of these trees were sprayed with water before and some after the fumigation. No injury occurred under these conditions, and the material was thought to be of sufficient importance to justify resuming work on the dust method of fumigating citrus trees.

In the manufacture of "C" calcium cyanide any percentage of HCN up to a certain maximum may be incorporated. The grade discussed here contains 30 per cent of HCN. A grade containing 50 per cent of HCN is also manufactured. The latter has not been used thus far for citrus fumigation, but it is included in some of the experiments described below. "C" calcium cyanide is manufactured by combining liquid HCN with calcium carbide.[18]4

"C" calcium cyanide is in a very fine state of division and is of a light brown color. The color is due to a slight polymerization that occurs, and this apparently increases as the amount of water used in the manufacture increases. Hydrocyanic acid gas comes off from the surface of the material rapidly enough to ignite. When the surface has sealed over, the gas is prevented from coming from below, but it will ignite again if a new surface is exposed.

Atmospheric moisture in contact with the cyanide produces hydrocyanic acid according to the following reaction:

$$CaH_2(CN)_4 + 2H_2O \rightarrow Ca(OH)_2 + 4HCN.$$

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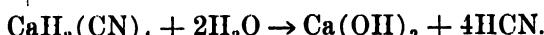
4 In the article referred to the following reactions are given:

$CaC_2 + 2H_2O = Ca(OH)_2 + C_2H_2$.

$Ca(OH)_2 + 2HCN = Ca(CN)_2 + H_2O$
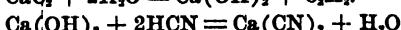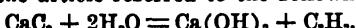
A slight amount of water (about 2 per cent calculated on the weight of the carbide) is added as a catalyst. The author states that "It appears that the reaction is not exactly in accordance with the equations given above, but rather may be summed up as

$CaC_2 + 4HCN \rightarrow Ca(CN)_2 . 2HCN + C_2H_2$

or in reality this reaction may be entirely analogous to the formation of calcium bicarbonate, i.e., the compound formed may be $CaH_2(CN)_4$."
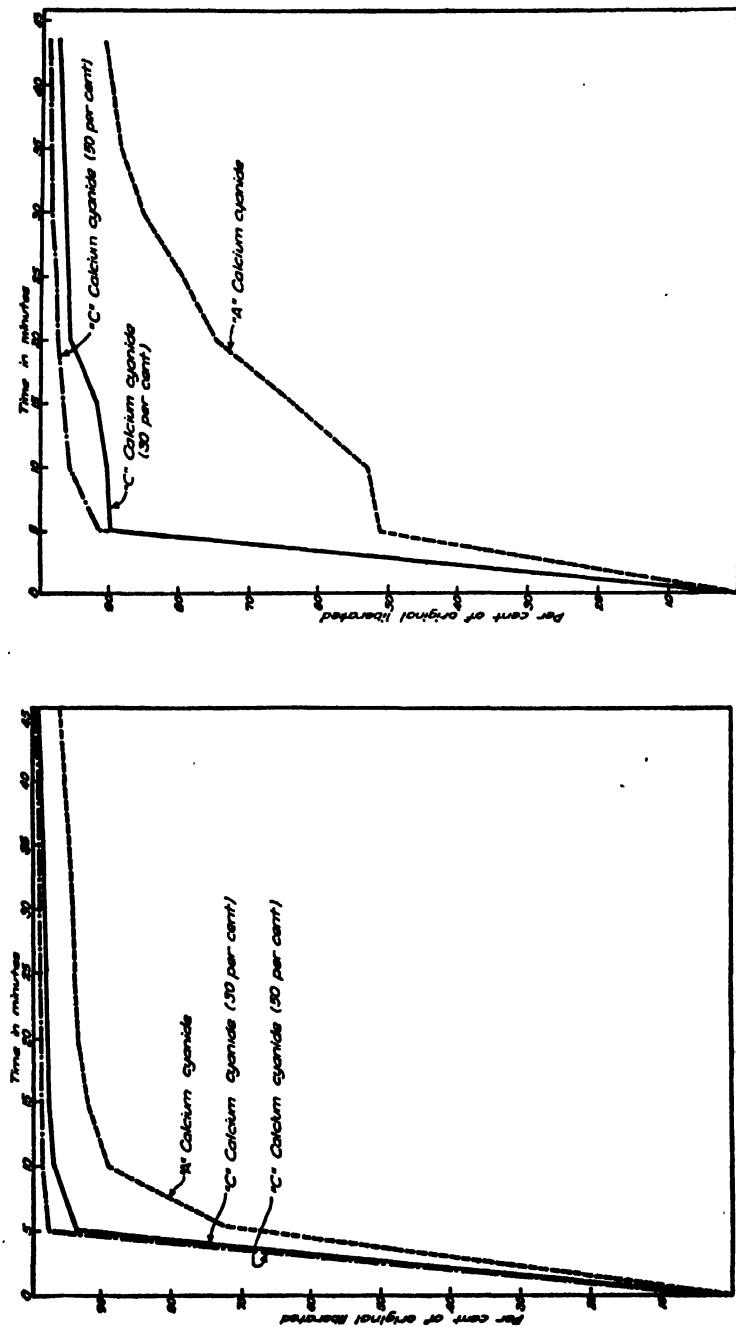
Fig. 1. The rate of evolution of hydrocyanic acid gas from calcium cyanide under canvas tents over form trees; A, with a relative humidity of 57-58 per cent and a temperature of 71°-74° F; B, with a relative humidity of 20-22 per cent and a temperature of 75°-77° F.

Practically all of the HCN will be liberated when the material is laid down in thin layers; the thinner the layer, obviously, the more rapid the evolution of the gas. This is the system followed in fumigating greenhouses and dwellings. For the purpose of this discussion, however, we are concerned only with the method followed in fumigating citrus trees, which consists of blowing the material under the tent by means of a dust blower. This method results in a more rapid evolution of the gas because the particles are separated and hence in more immediate contact with the moisture in the air. Blowing calcium cyanide dust into the air is a method more nearly standardized than laying the material on the floor, because it is impossible to lay down uniform layers.

RATE OF EVOLUTION OF HCN GAS

The rapidity of evolution of the gas when the cyanide dust was blown under a form tree[5] was determined by collecting the residue on papers at different intervals and analyzing it for the percentage of HCN remaining.

The graph, figure 1A, shows the evolution of HCN gas from "A" calcium cyanide, "C" calcium cyanide, and "C" calcium cyanide (50 per cent), at temperatures of 71 degrees to 74 degrees F and relative humidity of 57 to 58 per cent. At the end of 5 minutes 72.7 per cent of the HCN was liberated from "A," 93.2 per cent of the HCN from "C," and 97 per cent of the HCN from "C" (50 per cent). At the end of 10 minutes 89 per cent of the HCN was liberated from "A," 96.4 per cent from "C," and 98 per cent from "C" (50 per cent). At the end of the 45-minute period 95.8 per cent of the HCN in "A" was liberated, 99 per cent of the HCN from "C" and 99.1 per cent of the HCN from "C" (50 per cent).

The same experiment was also carried out (figure 1B) with a much lower humidity, from 20 to 22 per cent as against from 57 to 58 per cent in the first determinations. The temperature was not greatly different. Thus far no difference in the rate of evolution of the gas corresponding to difference in temperature, has been noted within the range of temperature at which commercial citrus fumigation is practical, or between 40 degrees and 80 degrees F. Referring to figure 1B, at the end of 5 minutes, 50.8 per cent of the HCN was liberated from

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[5] The form tree consisted of a wooden framework built in the shape of an orange tree of ordinary size, covered by a fumigating tent. The size was 26 feet over the top, with a circumference of 31 feet.

"A," 90.2 per cent from "C," and 91.6 per cent from "C'" (50 per cent). At the end of 10 minutes 52.7 per cent of the HCN was liberated from "A," 90.6 per cent from "C," and 96.0 per cent from "C'" (50 per cent). At the end of the 45-minute period 91.0 per cent of the HCN was liberated from "A," 98.3 per cent from "C," and 99.0 per cent from "C'" (50 per cent).

The very low relative humidity, from 20 to 22 per cent, did not greatly retard the evolution of gas from "C" calcium cyanide, but it affected a marked retardation in the evolution of the gas from "A" calcium cyanide, although by the end of the 45-minute period a fairly good percentage (91 per cent) had evolved. However, no commercial citrus fumigation is carried on when the humidity is as low as 22 per cent. The time of the experiment was 6 P.M. Such humidity occurs during the north winds or so-called northers in southern California.

COMPARATIVE DOSAGE OF "C" CALCIUM AND LIQUID HCN

In any new system of fumigation one of the most important questions is the matter of dosage. This point was investigated by the effect on the insects as well as the determination of concentration of gas in a fumigatorium and under tented citrus trees at different intervals, in comparison with liquid HCN. In our first experiments in the use of calcium cyanide in dust form, it was observed that it was not necessary to have the dust carry as much HCN as was used in liquid form to secure the same effect on the scales. This fact was explained at the time by assuming that a smaller amount of gas was necessary because the dust particles were thoroughly distributed over the tree and that the generation of gas took place from each of these particles, many of which were in close contact with the scales, and thus the potency of the gas might be increased. When the dust was simply blow on the ground or not well distributed over the tree a greater dosage was necessary. This fact tended to support the idea that the diffusion of the dust particles is important. It has also been shown that young scale insects (crawlers) when in close proximity to particles of dust cyanide in the open will be killed by the gas evolved. It kills other insects that are less resistant than scale insects in a similar manner.

When a study was made of the gas concentration under the tent it was found that the same concentration occurred when less actual

HCN was put under the tent with the dust than with the liquid. This could be accounted for only by the fact that less gas must escape through the tent in one case than in the other. The data presented in figures 4 to 9 seem to support this contention. When the fumigation is conducted in an enclosure that does not permit any leakage, this differential in favor of the dust does not appear.
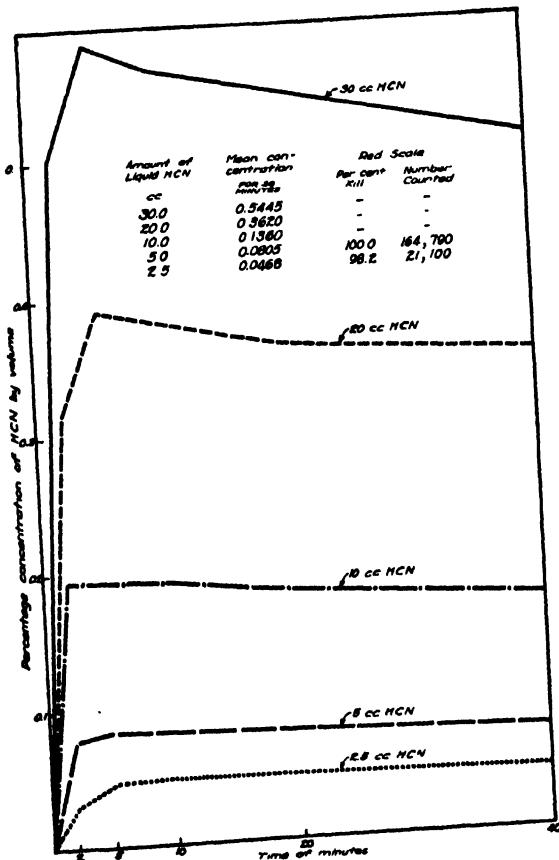
Fig. 2. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, from different amounts of liquid hydrocyanic acid in a fumigatorium of 100-cu.-ft. capacity. The temperature was 62 F and the relative humidity 33 per cent. Compare with figure 3.

Equivalent amounts of HCN must be carried in the dust and the liquid in a gas-tight fumigatorium in order to secure the same concentration of gas within.
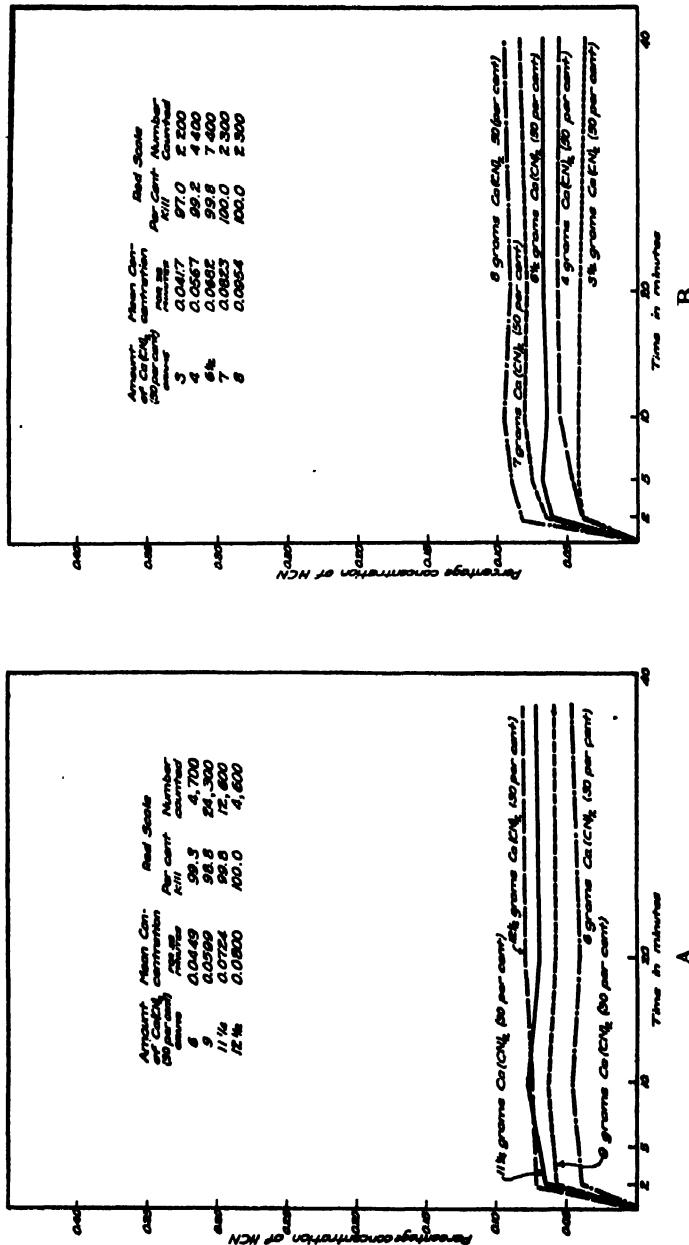
Fig. 3. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, in a fumigatorium of 100-cu.-ft. capacity; A, of different amounts of "C" calcium cyanide; B, of different amounts of "C" calcium cyanide (50 per cent). Compare with figure 2.

COMPARISONS OF "C" CALCIUM CYANIDE, "C" CALCIUM CYANIDE
(50 PER CENT) AND LIQUID HCN (98 PER CENT) ·
IN FUMIGATORIUM

From the figures in table 1 it will be seen that the given amounts
of the two grades of Ca(CN)₂ carried approximately as much HCN
as was carried in 5 cc of liquid HCN, and that the mean concentration
in the percentage of HCN as recovered in the fumigatorium were
approximately equal.

TABLE 1

LIQUID HCN AND "C" CALCIUM CYANIDE; CONTENT OF HCN AND MEAN
CONCENTRATION OF HCN GAS PRODUCED IN FUMIGATORIUM
OVER A 38-MINUTE PERIOD

Material	Amount	Amount of HCN	Concentration of HCN gas (percentage determined in fumigatorium)
		grams	
Liquid HCN........................ (98 per cent)	5 cc	3.415	0.0805
Calcium cyanide............ (30 per cent HCN)	12.5 grams	3.750	0.0800
Calcium cyanide............ (50 per cent HCN)	7 grams	3.500	0.0823

COMPARISONS IN FIELD TESTS WITH CANVAS COVERS OVER
· CITRUS TREES

Description of Methods.—Samples of HCN gas were taken from
three points in the tree: one about one foot from the top of the tent,
one from the center, and one one foot from the ground close to the
tree trunk. Aluminum tubes (in a few cases copper tubes with shellac
on the outside and inside were used) were placed at the intake posi-
tions. The ends of these tubes were loosely plugged with cotton to
prevent particles of dust from entering the system in the case of
Ca(CN)₂ and also in the case of the liquid to impose the same
conditions. From the other end of the metal tubes, rubber tubes
conducted the gas, underneath the margin of the tent, to the aspiration
bottles.

Fig. 4. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, after dosages of liquid hydrocyanic acid and of "C" calcium cyanide; A, after a 20-cc dosages of liquid hydrocyanic acid (98 per cent); B, after a 1¼-ounce dosage of "C" calcium cyanide (30 per cent). The fumigating was done under canvas tents over citrus trees. The tests were conducted on November 15, 16, and 17, 1926, with temperatures of 65°, 67°, and 54° F, and relative humidities of 28, 30, and 48 per cent, respectively. Top, center, and bottom of the tent are indicated in the chart by *t*, *c*, and *b*.

The averages of six field tests of each fumigant are presented.

*A 20-cc dosage means that 20 cc of liquid HCN is used for each unit called for in the dosage schedule in general use in citrus fumigation in California. For example, a tree measuring 30 feet over the top and 36 feet in circumference requires 10 units. With a 20 cc schedule, therefore, 200 cc of liquid HCN would be used. With an 18 cc schedule 180 cc would be used. In the tests with calcium cyanide a 1¼ ounce dosage means 1¼ ounces for each unit called for in the dosage schedule.

Fig. 5. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, after dosages of liquid hydrocyanic acid and of calcium cyanide; A, after a 20-cc dosage of liquid hydrocyanic acid (98 per cent); B, after a 1¼-ounce dosage of "C" calcium cyanide (30 per cent). The fumigating was done under canvas tents over citrus trees. The averages of four field tests of each fumigant are presented. The tests were conducted on November 18 and 19, with temperatures of 69° and 60° F, and relative humidities of 64 and 49 per cent, respectively. Top, center, and bottom of the tent are indicated in the chart by t, c, and b.

When the tubes had been installed as described, the tents were pulled over the tree, the dosage required for each tree was determined by measurement and the charge administered. Aspirations were made simultaneously from the three points in the tree at intervals of 2, 5, 10, 20, and 40 minutes from the time when the charge was completed. When liquid HCN and $Ca(Cn)_2$ dust were compared the second tree was charged six minutes after the first, this interval being found most suitable for the manipulations and short enough to insure practically the same external conditions for the two trees. In so far as possible trees of approximately the same size were chosen. In one series of experiments the same four trees were fumigated once each night for two weeks and the liquid and dust alternated each time. The intake tubes were left in the same position so that the two methods were compared under conditions as nearly identical as possible.

To start the aspiration, the water valves B and B' (fig. 6) were opened; this allowed the water to pass from A to A'. As the water ran out of A a vacuum was created, which in turn created a vacuum in C; this pulled the air through D from the intake position in the tree. The hydrocyanic acid taken in with the air was absorbed by the sodium carbonate in the aspiration bottle C. The volume of air and HCN taken out was determined by the volume of water in the aspiration can A which in these tests was three liters. About one and one-half minutes were required for the water to run from A to A'. The aspiration being complete the two-hole stopper in C was immediately removed and placed in the next bottle, and a solid rubber stopper put in its place. The water valve B was closed and the rubber tube E was removed from A and attached to A'. The positions of the aspiration cans A and A' were reversed and thus the set-up was made ready for the next aspiration. Immediately after the completion of the aspiration the titrations were made in which iodine solution was used to determine the end point.

Computations.—The following computations which were used in this work were kindly furnished by E. R. Hulbirt, of the Owl Fumigating Corporation, Azusa, California.

The essential chemical reaction involved in the titration is

$$I_2 + HCN = HI + ICN.$$
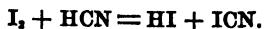
A single drop of the iodine solution will cause the starch indicator to assume a dark blue color at the end point. A blank titration should be made, and from the results of this, the proper correction made in the volume of iodine solution used in each cyanide determination.

For a N/20 iodine solution, 1 cc is equivalent to 0.000675 grams of HCN. Then the percentage of HCN by volume in the aspirated sample will be

$$\frac{0.000675 \times (\text{cc iodine sol.}) \times 900 \times 100}{3000}$$

where 3000 is the volume of the aspiration sample, and 900 is the volume in cubic centimeters of 1 gram of HCN.

Both the 900 and 3000 are used without temperature and pressure corrections, for it is obvious from the formula that such corrections would cancel. Further simplified, the formula becomes 1215 N/cc where N = normality of the iodine solution used, and cc = the number of cubic centimeters in the aspiration sample. This becomes a constant for the same iodine solution and a fixed aspiration volume. Hence, to determine the percentage of HCN by volume in a gaseous sample, merely multiply the number of cubic centimeters of standard iodine solution used in the titration by the constant.
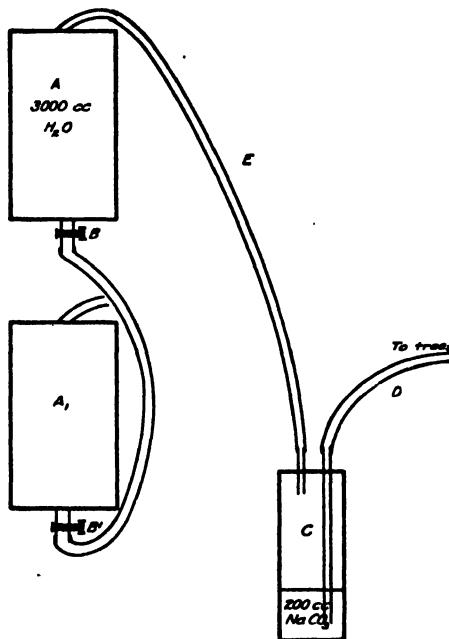
Fig. 6. Sketch showing how samples of hydrocyanic acid gas were aspirated from within the tent.

From figures 4, 5, 7, and 8 it will be noted that 1¼ ounces of "C" calcium cyanide is about the equivalent of 20 cc of liquid HCN (98 per cent). Also (fig. 9) that 1 ounce of "C" calcium cyanide is about the equivalent of 16 cc of liquid HCN. That is, the mean concentration of gas under a tented citrus tree at night is approximately the same when the calcium cyanide and liquid are used in the ratios as given above. As judged also from the results on the scale insects these ratios are equivalent. The aspirations here given represent less than one-fourth of the total aspirations made. Each graph represents
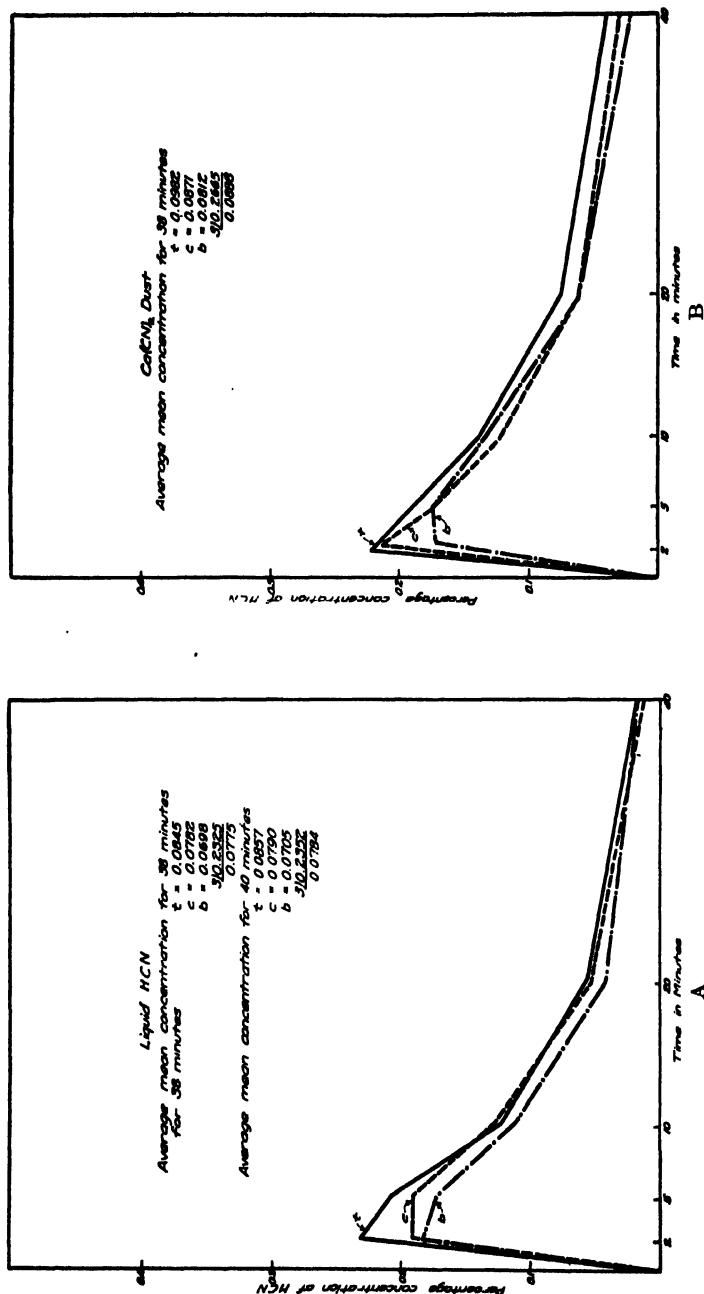
Fig. 7. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, after dosages of liquid hydrocyanic acid and of calcium cyanide; A, after a 20-cc dosage of liquid hydrocyanic acid (98 per cent); B, after a 1¼-ounce dosage of "C" calcium cyanide (30 per cent). The averages of three field tests of liquid hydrocyanic acid and of six field tests of calcium cyanide are presented. The fumigating was done under canvas tents over citrus trees. The tests were conducted on October 20 and 22, 1926, with temperatures of 58° and 57° F, and relative humidities of 77 and 88 per cent respectively. Top, center, and bottom of the tent are indicated in the chart by t, c, and b.
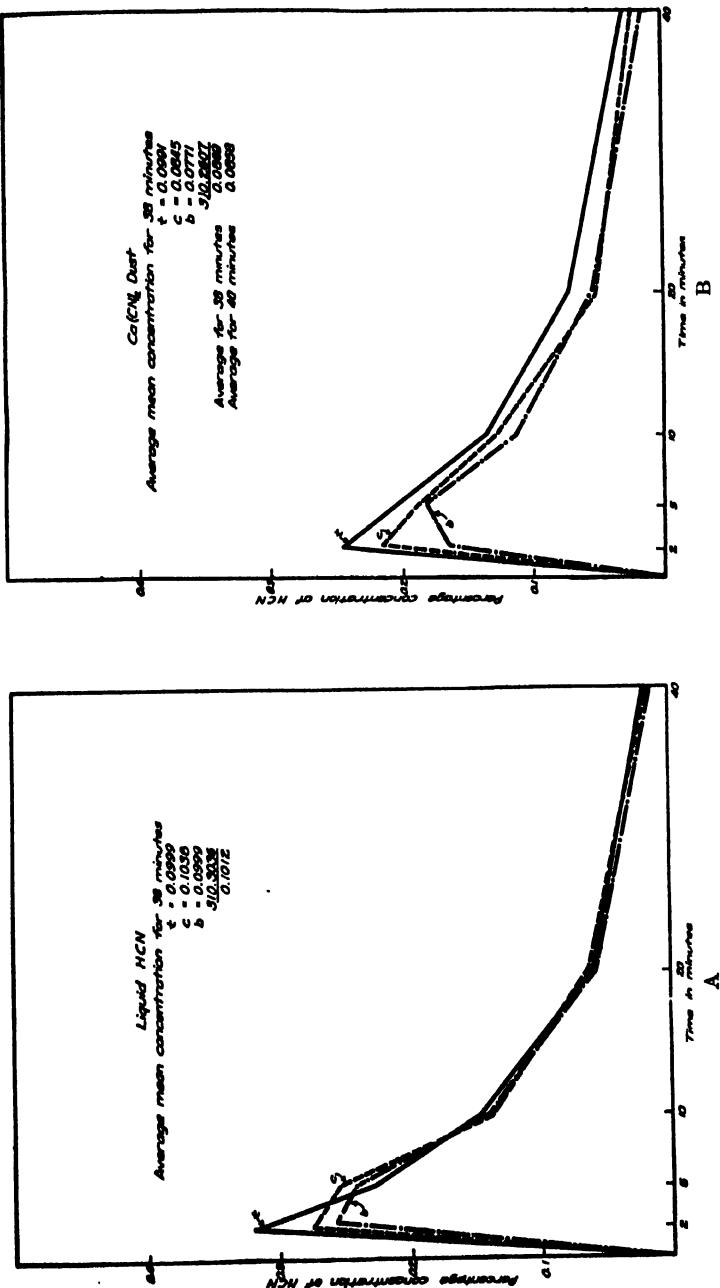
Fig. 8. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, after dosages of liquid hydrocyanic acid and of calcium cyanide; A, after a 20-cc dosage of liquid hydrocyanic acid (98 per cent); B, after a 1¼-ounce dosage of "C" calcium cyanide (30 per cent). The fumigating was done under canvas tents over small lemon trees. The tests were conducted in Orange County on October 25 and 29, 1926, with temperatures of 54° and 58° F, and relative humidities of 94 and 77 per cent, respectively.
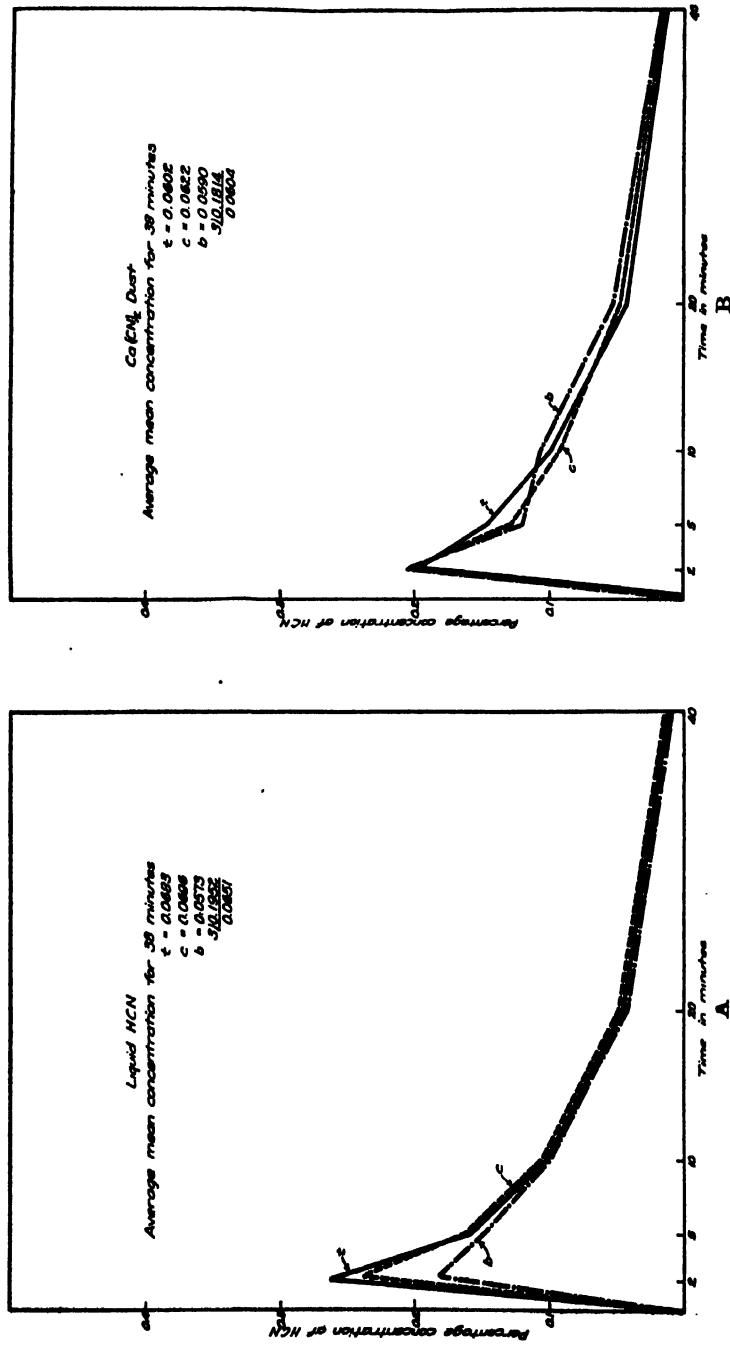
Fig. 9. The concentration of hydrocyanic acid gas at different intervals, and the mean concentration for 38 minutes, after dosages of liquid hydrocyanic acid and of calcium cyanide; A, after a 16-cc dosage of liquid hydrocyanic acid (98 per cent); B, after a 1-ounce dosage of "C" calcium cyanide (30 per cent).

The averages of four field tests of each fumigant are presented. The fumigating was done under canvas tents over citrus trees. The tests were conducted on November 22 and 23, with temperatures of 57° and 64° F, respectively, and a relative humidity of 66 per cent (both days). Top, center, and bottom of the tent are indicated in the chart by *t*, *c*, and *b*.

the average of a set of aspirations which were made under different conditions as to temperature and relative humidity. In every case, however, the dust and liquid cyanide were compared under the same conditions. Some determinations have been made with larger amounts, or 1½ ounces of calcium cyanide in comparison with 24 cc of liquid. These ratios should be equivalent so far as gas concentration is concerned, but there is some evidence tending to indicate that with high concentrations the HCN in the air may be in equilibrium
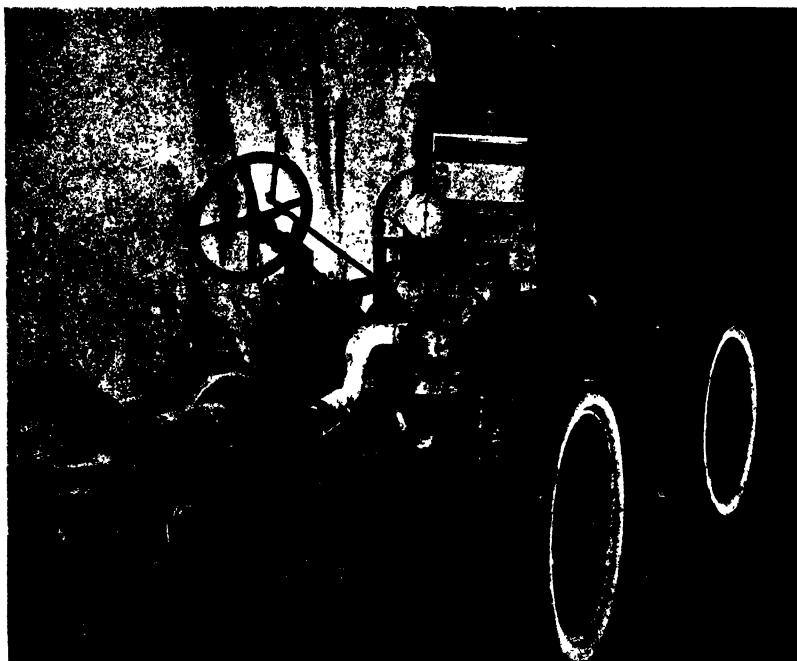
Fig. 10. Motor-power dust applicator for applying cyanide dust under tented trees.

with that in the dust and thus prevent complete evolution of the gas. The mean concentration was calculated for the most part for a 38-minute interval, or from 2 minutes after the charge to 40 minutes after. The average for the concentrations at 2 minutes and at 5 minutes was multiplied by the time interval of 3 minutes. Likewise the average for 5 minutes and 10 minutes was multiplied by the 5-minute interval, and so on through the period, when the total was divided by the total time interval, or 38 minutes. Averages were obtained also for the 40-minute interval, starting from the time the charge was given; in this case one-half the concentration at 2 minutes was included and the whole divided by 40 instead of by 38. This gave

a slightly lower mean concentration and may be preferable to the 38-minute interval. The writer would be inclined in future work to base the mean concentration on the full period from the time when the charge was given, which in this case would be 40 minutes.

In order to secure approximately the same gas concentration under a canvas tent as well as the same effect on insects, one should use an amount of calcium cyanide that carries about one-fourth less HCN by weight than is carried in the equivalent amount of liquid HCN. For example, 20 cc of liquid contains 13.6612 grams of HCN; one-fourth or 3.4153 grams less than this = 10.2459 grams or approximately the amount (10.6311 grams) contained in 1¼ ounces of calcium cyanide.

Another Method of Determining Mean Concentration.—Carrying on aspirations in the field is a tedious task, and the number of trees from which tests may be made is limited. Sixty bottles are required for four trees where five aspirations are made from each of three points. In an attempt to simplify this procedure a single bottle containing Na_2CO_3 was placed in the top, another in the center, and a third at the bottom of each tree, and allowed to remain throughout the fumigation period, when they were immediately removed and stoppered. They must be suspended vertically, otherwise the surface area of the liquid in the jars will vary, and consequently the absorption of the gas will vary. While we were comparing the concentration of gas from the dust it was necessary to cover the mouths of the bottles with two or three layers of cheesecloth and this same condition was imposed on the bottles where liquid HCN was used. When a considerable amount of the dust settles on the cheesecloth, as was occasionally the case, too much gas was absorbed. The method was abandoned, therefore, in making comparisons where the dust was involved. For comparisons of the mean concentration of gas from the liquid the method seemed to work satisfactorily. It was checked with the standard aspiration method, and the results agreed fairly closely. In most of such work after the state of diffusion at different intervals is once known, the matter to be determined is the mean concentration rather than the concentration at particular intervals. Bottles containing alkaline solution so placed in the tree absorb the gas throughout the period of exposure. When these are titrated the percentage of gas can be determined and this without further calculation would represent the mean concentration. The temperature of the absorbing material makes a difference, so that the method is applicable only for a comparison of two or more trees fumigated at the same time and place.
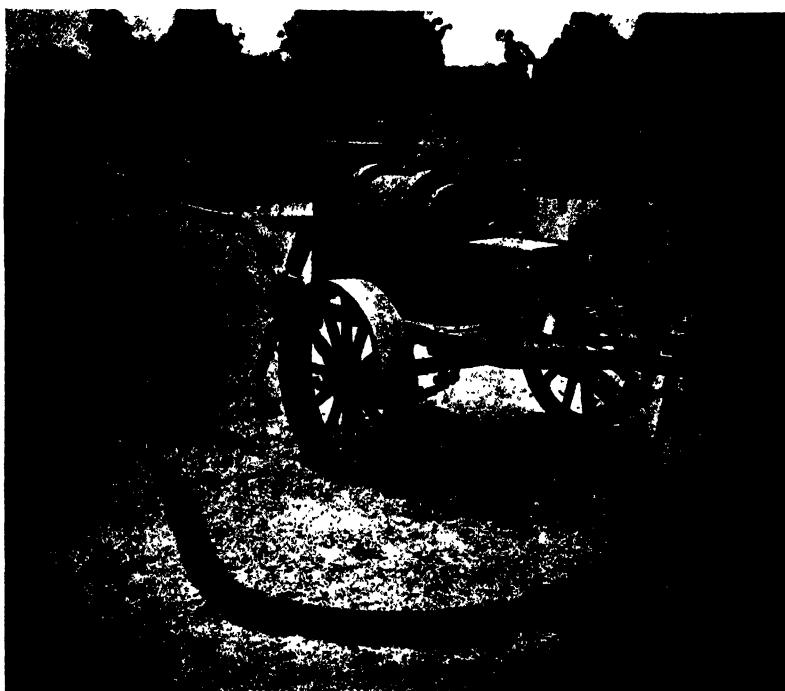
Fig. 11. Horse-drawn power dust applicator for applying cyanide dust under tented trees. A motor operating an air pump forces the material under the tent.

Fig. 12. Hand dust applicator for applying cyanide dust under tents or enclosures.

TABLE 2

Summary of One Set of Field Tests

Liquid HCN			Ca(CN)₂		
Number of tests	Dosage	Mean concentration liquid HCN	Number of tests	Dosage	Mean concentration HCN
	cc	per cent		ounces	per cent
21	20	0.0841	16	1¼	0.0825
4	16	0.0651	4	1	0.0604

TABLE 3

Grams HCN is Given Amounts of "C" Calcium Cyanide (30-per-cent HCN) and Liquid HCN (98 per cent)

"C" Calcium cyanide	HCN	Liquid HCN	HCN
ounces	grams	cc	grams
¼	2.1262	12	8.1967
½	4.2524	14	9.5628
¾	6.3786	16	10.9290
1	8.5049	18	12.2951
1¼	10.6311	20	13.6612
1½	12.7573	22	15.0273
1¾	14.8835	24	16.3934
2	17.0097	26	17.7596

DIFFUSION OF GAS UNDER THE TENT WHEN GENERATED FROM CALCIUM CYANIDE

When cyanide is applied in dust form under a tented tree the initial diffusion of the gas is largely dependent on the manner in which the dust is distributed. If the dust is placed wholly on the ground, the bottom of the tent will carry the heaviest concentration, at least for a short period. If the dust is blown well to the top of the tree, the concentration will be greatest at this point and will remain so because of the tendency of the gas to rise. In the case of trees of small to medium size, with the machines in present use, the gas occurs in slightly greater concentration at the center and top of the tree. The aspirations also show a slightly greater concentration of gas from the dust at the end of 40 minutes than from the liquid. This may be accounted for by the fact that the gas continues to generate longer from the dust than from the liquid.

METHOD OF APPLYING CALCIUM CYANIDE DUST
UNDER THE TENTED TREE

Calcium cyanide is transported to the field in cans having a friction top which excludes the air. The friction lid is removed and a false top put in place when the container is inverted over the hopper of the machine (see fig. 10), and a sliding lid is removed to allow the material to drop into the hopper. In this way very little air comes in contact with the cyanide until it is blown under the tent. A crank operating a screw in the bottom of the hopper feeds the material into the weighing device until the proper dosage for the tree may be read on the dial. An air pump which is operated by the automobile engine (fig. 10), or by a gasoline engine (fig. 11) supplies the air which blows the charge through a large hose whose open end has been previously placed under the tent. A nozzle on the end of the hose spreads the charge and directs it upward through the tree. For small trees or where the work is not too extensive a hand machine (fig. 12) may be used. The same weighing device is installed as on the larger machine, and the foot bellows furnishes the force for distributing the charge under the tree.

SUMMARY

1. The recent production of calcium cyanide $(Ca(CN)_2)$, a less stable compound than the sodium cyanide $(NaCN)$ or potassium cyanide (KCN) heretofore used for fumigation purposes, has made it possible to employ a different method of fumigation which consists of simply blowing the material or putting it down in thin layers in finely divided form into an enclosure.

2. The atmospheric moisture acting on the small dust particles produces a sufficiently rapid generation of gas to make the method applicable even to citrus fumigation, where the fumigation period does not exceed one hour.

3. Two forms of calcium cyanide designated in this paper as "A" calcium cyanide and "C" calcium cyanide are discussed.

4. "A" calcium cyanide, in powdered form, first used by the author in 1922, is largely used for citrus fumigation and for rabbits in Australia, for greenhouse and other fumigation generally, as well as for dusting in the open (without covers) for several insect pests.

5. The investigation of "A" calcium cyanide for citrus fumigation in California was terminated in 1924 because of injury to the citrus tree.

6. In 1925 "C" calcium cyanide was first tried for citrus fumigation in California and the injury resulting from the residue of "A" calcium cyanide was practically entirely obviated with this material.

7. "C" calcium cyanide was used in a considerable amount of commercial citrus fumigation in the state during 1926.

8. Comparisons of dosage between "C" calcium cyanide and liquid HCN are given; the determinations are based on the effects on insects and on the actual gas concentration under the tent at different intervals.

9. Less HCN is required in the calcium cyanide dust than in liquid HCN to effect the same mean concentration of gas under a canvas cover.

10. In the case of a gas-tight fumigatorium the same amount of HCN must be carried in the dust as in the liquid to give the same mean concentration within.

11. From the last two facts it is concluded that there is less escape of gas through canvas covers where the source of the gas is the dust than where the source of gas is the liquid HCN.

12. About 25 per cent less gas is required in the dust than in the liquid. The data given indicate that $1\frac{1}{4}$ ounces of "C" calcium cyanide dust is equivalent to 20 cc of liquid HCN, and there is approximately 25 per cent less HCN in this amount of the dust than there is in 20 cc of HCN.

13. The evolution of gas from "C" calcium cyanide was not greatly retarded when the relative humidity was as low as 20 to 22 per cent, but this humidity did markedly retard the evolution of gas from "A" calcium cyanide. The evolution of gas from both cyanides seemed to be independent of temperature within ordinary fumigation limits, that is, between 40 and 80 degrees F.

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THE INHERITANCE OF FLOWER TYPES IN CUCUMIS AND CITRULLUS

J. T. ROSA[1]

SEX FORMS IN FRUIT-PRODUCING FLOWERS

The horticultural varieties of melons and cucumbers, belonging to the genus *Cucumis*, and of watermelons, belonging to *Citrullus*, may be divided into two groups according to the arrangement of the sex organs. In one, the andromonoecious group, the plants bear staminate and hermaphrodite flowers. The former are borne in clusters of five in the axils of the main axis and of the lateral branches, while the latter occur singly on the two basal nodes of lateral branches of the first and second order (Rosa,[8], [9]). In the second, or monoecious group, the plants bear staminate and pistillate flowers, the location of the pistillate being the same as that of the hermaphrodite flowers in group 1. Monoecism generally is said by systematists to be the typical condition in *Cucumis* and *Citrullus*. However, the occurrence of hermaphrodite flowers in cantaloupe melons has been reported comparatively recently by Munson,[6] and Blinn,[1] and in watermelons by Rosa.[9] A typical hermaphrodite flower of the cantaloupe melon is illustrated in figure 1.

In cultivated varieties of melons (*Cucumis melo*) of the present day, the andromonoecious group greatly predominates in number. All of the varieties of English and American netted melons (*C. melo* var *reticulatus*) which were grown at Davis in 1923, 1924 and 1925 were found to have hermaphroditic flowers. About 300 different varieties of this group came under observation. In 1925, however, the Snake melon (*C. melo* var. *flexosus*) and three European varieties

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of netted melon, Sucrin de Tours, Heinemann's Freiland, and Japan White, were found to be producing pistillate flowers. In 1926, 84 varieties of melons from various European and Asiatic sources were grown at Davis, and of these 76 were andromonoecious, 7 were monoecious, and in 1 some plants belonged to one group, some to the other. In 1927, 63 new foreign varieties were tested; 56 were andromonoecious, 5 were monoecious and 2 were mixed.

Cucumis cocomon, of which several varieties were grown, is andromonoecious. *C. utilissima* and *C. mormodica* are monoecious. The three foregoing cross readily with *C. melo* and should be considered merely as botanical varieties of that species, according to the delimitations established by Naudin. *Cucumis anguria,* the West Indian Gherkin, is monoecious; attempts to cross it with *C. melo* and with *C. sativus* failed.

In the cucumber (*Cucumis sativus*), conditions are different from those in melons. Of about 50 American and European varieties which have been tested, all are monoecious except one, the Lemon cucumber, which has hermaphroditic flowers.

In watermelons and citron melons (*Citrullus vulgaris*) the monoecious condition predominates. Of about 60 varieties which have been tested at Davis, only a few have been found to possess hermaphroditic flowers. These include the Black Seeded Angelino, Black Seeded Chilian, Snowball, Winter King, and a Kalihari citron melon.

The monoecious condition in the plant kingdom in some instances appears to be unstable, the exact forms of sex expression tending to be altered by environmental conditions. Thus, Higgins and Holt[4] report a variety of forms of sexual expression in the papaya (*Papaya carica*); and Schaffner[11] produced apparent cases of sex reversal in *Arisaema,* through manipulation of moisture and nutrition. It is also of especial interest that Durham[2] found hermaphroditic flowers in certain inbred lines of the summer squash, *Cucurbita pepo,* which has always been considered as strictly monoecious. Moreover, the hermaphroditism observed by Durham in squash appears to have been, at least in some cases, of a transitory nature, as some of the plants returned to the monoecious condition in the latter part of the season.

In contrast to the instances mentioned above, sexual forms in *Cucumis* and *Citrullus* appear to be remarkably constant. The basis for this statement is found in the writer's pollination records for 1924–1927. During the course of breeding work with melons, cucumbers and watermelons, the sex conditions in the flowers employed have been recorded. In the cucumbers and watermelons, out of several

hundred flowers of each that have been examined, no case of her-
maphroditic flowers on monoecious varieties or plants has been found.
However, in the andromonoecious varieties of watermelon, some varia-
tions do occur. Thus, hermaphroditic flowers are found occasionally,
especially toward the end of the flowering period, with only one or
two stamens instead of three. But the missing stamens are usually
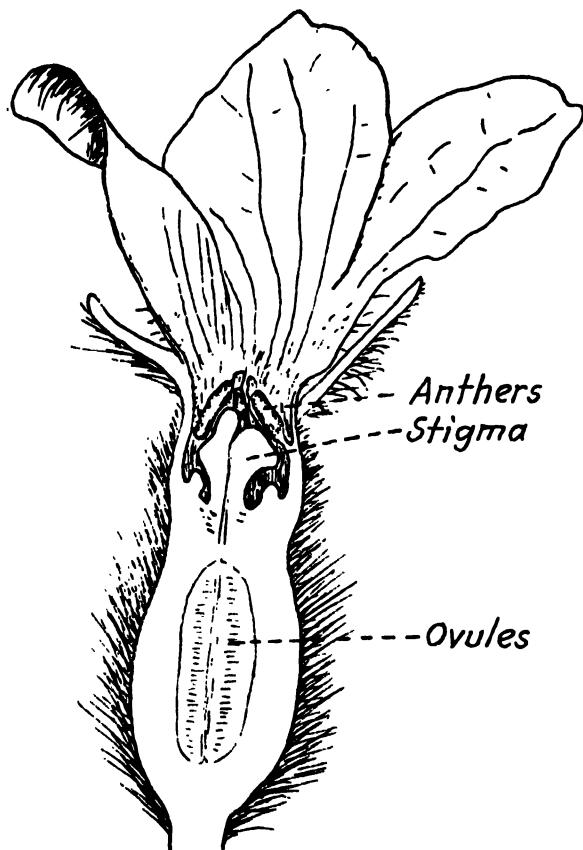represented by more or less well-developed staminodia.

Fig. 1. Longitudinal section of hermaphroditic flower, Salmon Tint melon.
The stamens are attached near the base of the corolla tube.

On the andromonoecious plants of some varieties of *Cucumis melo*,
occasional pistillate flowers have been found. Table 1 gives a sum-
mary of the observations on flowers of cantaloupes and related varie-
ties of melons. Nearly half of the flowers recorded were in inbred
lines of the variety Salmon Tint, the others being in miscellaneous
varieties of *C. melo* which have been used in the breeding work.

TABLE 1

SEXUAL FORMS OF FLOWERS OBSERVED IN *Cucumis* melo.

Year	Andromonoecious plants		Monoecious plants	
	Hermaphroditic flowers	Pistillate flowers	Hermaphroditic flowers	Pistillate flowers
1924	200	0	0	0
1925	542	1	0	30
1926	955	6	0	60
1927	1090	8	0	158
Total........	2787	15	0	248

   In monoecious melon plants no case of reversal to the prevailing pistillate condition has been observed, though it is not possible to say that such reversal never occurs, as the number of flowers observed is not very large. With the andromonoecious varieties, however, 15 instances of reversal in 2,802 flowers, or 1 in 187, were found. As an average plant may produce about 60 hermaphroditic flowers during its chief flowering period, this indicates that about one plant in three may produce a single flower of aberrant sex form. The production of the aberrant forms has not been observed to be correlated with any particular environmental conditions, nor to any certain stage of plant development. The seed from pistillate flowers borne on andromonoecious plants, when pollinated by staminate flowers of the same plant, give rise in the following generation to andromonoecious plants, with flowers and fruit like the prevailing type of the parent. No explanation of the occurrence of the rare pistillate flowers is available, other than the supposition that they result from some circumstance in the ontogeny of the flower, which leads to the omission of the stamens. They may be the result of a somatic mutation, which does not affect the cell layers from which the ovules arise, hence are not transmitted to progeny.

   An interesting fact is that the fruits which develop from pistillate flowers on prevailing andromonoecious plants, are different from those arising from hermaphroditic flowers on the same plant. These differences are in part shown in figure 2. The fruit from the pistillate flower is larger, longer, has a much lower ratio of width to length, and shows the longitudinal sutures much more plainly, than fruits from

hermaphroditic flowers. In the Salmon Tint variety, five fruits known to have come from pistillate flowers have been observed, and they all consistently showed the differences in form just mentioned. As individual fruit measurements have been made on many fruits of inbred lines of Salmon Tint, it is possible to determine the frequency of occurrence of pistillate flowers in that variety by inspection of the fruit measurement records. In 1926 and 1927, 6,400 fruits were measured, and 16 showed the abnormal shape characteristics of fruit from pistillate flowers. In this variety, then, the frequency of pistillate flowers appears to be about 1 in 400, of those that actually produce fruit.
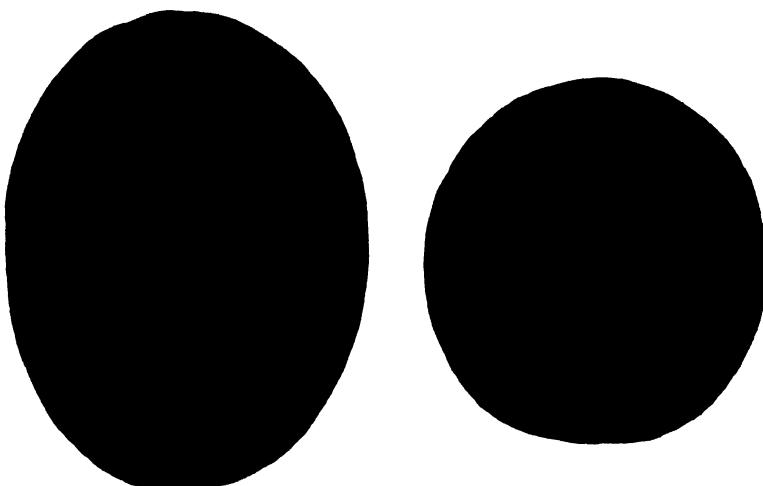
Fig. 2. Two fruits from the same plant of Salmon Tint melon. The one on the left came from a pistillate flower, the one on the right from an hermaphroditic flower. The latter fruit represents the normal for fruits of this particular inbred line.

Since the forms of sexual expression in melons, cucumbers and watermelons are subject to comparatively little fortuitous variation, it was considered of interest to investigate further the basis for the distinction of the two types which have been described. This is of importance not only in connection with other studies on the genetics of these genera, but because of the bearing on the general problems of evolution and of inheritace of sex expression in plants. Monoecious species have hitherto received rather little attention in studies on inheritance of sex.

Inheritance of Sex Forms

The selfing of andromonoecious varieties of melons in 1924, (*a*) with pollen from anthers in hermaphroditic flowers, and (*b*) with pollen from staminate flowers on the same plant, gave identical results in the progeny, i.e., the production of andromonoecious plants like the parent.

In 1925, a number of crosses were made between varieties of the two contrasted types of sex expression. The following is a list of the crosses which have been followed through the F_2 generation:

Ovule parent	Pollen parent
Melon:	
Salmon Tint (*andromonoecious*) ×	Freiland (*monoecious*)
Salmon Tint (*andromonoecious*) ×	Snake (*monoecious*)
Snake (*monoecious*) ×	Salmon Tint (*andromonoecious*)
Serpent (*monoecious*) ×	Salmon Tint (*andromonoecious*)
Salmon Tint (*andromonoecious*) ×	Waldemar Gratscheff (*monoecious*)
Salmon Tint (*andromonoecious*) ×	Sucrin de Tours (*monoecious*)
Cucumber:	
Lemon (*andromonoecious*) ×	Chicago Pickle (*monoecious*)
Lemon (*andromonoecious*) ×	Long Green (*monoecious*)
Watermelon:	
Conquerer (*monoecious*) ×	Angeleno (*andromonoecious*)
Klondyke (*monoecious*) ×	Angeleno (*andromonoecious*)
Green Seeded Citron (*monoecious*) ×	Angeleno (*andromonoecious*)
Angeleno (*andromonoecious*) ×	Green Seeded Citron (*monoecious*)

The parental plants of Salmon Tint and Klondyke used in the crosses were of second generation inbred lines. The parental plants of the other varieties were all from commercial stocks. That these parental plants were all homozygous for their respective sex forms was proved by growing progenies from selfed seeds of them in the following two years.

The F_1 generation of these crosses was grown in 1926. In all cases the monoecious form appeared to be completely dominant, the fruit-producing flowers being always pistillate. Back crosses were made to the presumably recessive hermaphroditic-flowered parents.

Results with Melons in the F_2 Generation.—The F_2 progenies were grown in 1927. During the middle portion of the flowering season, the pistillate flowers on the F_2 plants were examined by two men working independently. Their observations checked with each other in every case. Table 2 shows the distribution of sex forms which was found in the melon crosses.

TABLE 2

PHENOTYPES OCCURRING IN F_2 OF CROSSES BETWEEN MONOECIOUS AND
ANDROMONOECIOUS VARIETIES OF MELONS (*Cucumis melo*)

Cross	Number of plants	Plants with pistillate flowers	Plants with hermaphroditic flowers
Salmon Tint x Freiland......	74	56	18
Salmon Tint x Snake....	39	30	9
Snake x Salmon Tint...	135	109	36
Serpent x Salmon Tint...	39	29	10
Total....	**287**	**224**	**73**
Calculated, 3 : 1 ratio....	....	*222.75*	*74.25*

In the F_2 generation in the melon crosses there is a remarkably close approximation to a Mendelian mono-hybrid ratio, of three pistillate plants to one hermaphroditic-flowered. Accordingly, it may be concluded that hermaphroditism in melons depends upon a single recessive factor.

Results with Cucumbers.—The F_1 plants in the cucumber crosses always show the pistillate condition to be completely dominant. Only one small F_2 progeny was grown; this gave 11 plants bearing pistillate to 5 with hermaphroditic flowers. It is probable that in the cucumbers, as in melons, the hermaphroditic condition depends upon a single recessive factor.

Results with Watermelons in the F_2 Generation.—As with the other species, the F_1 plants of the watermelon crosses bore pistillate flowers. In the F_2 generation, segregation occurred as shown in table 3. The distinction between the phenotypes was not so clear cut as in melons and cucumbers, however. The stamens in the hermaphroditic flowers were not always equally well developed, but as mentioned previously, this condition is also found in the parental andromonoecious varieties. In some of the pistillate F_2 plants, small staminodia were observed occasionally. There was, however, no difficulty in classifying the plants.

Although only 113 plants were grown in F_2, the results are a nearly perfect 3:1 ratio, indicating that in watermelons also, the primary difference between pistillate and hermaphroditic flowers depends upon a single factor, hermaphroditism being recessive. However, it is possible, in watermelons, that this character may be more

sensitive to nutritional or environmental conditions, than it is in melons and cucumbers, as the stamens of hermaphroditic flowers are not always equally developed.

TABLE 3

PHENOTYPES OCCURRING IN F_2 OF CROSSES BETWEEN MONOECIOUS AND ANDROMONOECIOUS VARIETIES OF WATERMELONS (*Citrullus vulgaris*)

Cross	Number of plants	Plants with pistillate flowers	Plants with hermaphroditic flowers
Conqueror x Angeleno............................	33	23	10
Klondyke x Snowball	35	26	9
Klondyke x Angeleno	45	35	10
Total	113	84	29
Calculated, 3 : 1 ratio......	... ...	*84.75*	*28.25*

Results with the Back-crosses.—The F_1 hybrids were back-crossed to their recessive hermaphroditic-flowered parental variety. Table 4 shows the sex expression observed in the sesqui-hybrids.

TABLE 4

SEX EXPRESSION OF PLANTS FROM BACK-CROSSING F_1 PLANTS TO THEIR ANDROMONOECIOUS PARENT

Parentage		Number of plants	Plants with pistillate flowers	Plants with herma-phroditic flowers
Melons:	Salmon Tint x $\dfrac{\text{Salmon Tint}}{\text{Freiland}}$	40	23	17
	Salmon Tint x $\dfrac{\text{Salmon Tint}}{\text{Snake}}$	38	16	22
	Total...................	78	39	39
Watermelons:	Angeleno x $\dfrac{\text{Angeleno}}{\text{Citron}}$.......................	33	18	15 .
	$\dfrac{\text{Angeleno}}{\text{Citron}}$ x Angeleno.......................	27	13	14
	Total.......................	60	31	29

It is seen that the back-crosses with melons (*Cucumis melo*) happened to produce exactly the expected 1:1 ratio of pistillate and hermaphroditic plants. In the watermelons, the observed results likewise approach closely to the 1:1 ratio.

Discussion of Results on Sex Form.—The evidence both from F_2 progenies and from the back-crosses are in agreement, in indicating that the differentiation of pistillate and hermaphroditic flowers in *Cucumis* and *Citrullus* depends upon a single genetic factor, hermaphroditism being recessive. Moreover, the factor concerned in sex differentiation in this case must be borne both by micro- and macrogametes, since reciprocal crosses and back-crosses give concordant results.

The question may now be raised concerning the probable origin of the two forms of flowers in the Cucurbitaceae. Though the occurrence of hermaphroditic flowers has generally been overlooked by systematists, this form is probably not of recent origin. Thus, Spallanzani,[12] in the 18th century, found that some varieties produced fruit with viable seeds when the flowers were carefully isolated against the introduction of foreign pollen. And Sagaret[10] in 1824 found that the cantaloupe, Boul' de Siam, set fruit with viable seeds when the flowers were covered with bell jars. These writers suspected that the seeds they obtained were of parthenogenetic origin, but it is more likely that they resulted from the self fertilization of hermaphroditic flowers. Numerous tests by the present writer for parthenogenesis in various species of Cucurbitaceae have all given negative results. Phenospermic seeds occasionally are produced when pollen is not applied, or when incompatible pollen of another species is used.

From the evolutionary point of view, it is generally considered that the various plant families are advancing along parallel lines toward the dioecious condition (Yampolsky[14]). On this basis, the andromonoecious condition, with its hermaphroditic flowers, would be more primitive than the strictly monoecious form. The monoecious form in the Cucurbitaceae probably has arisen from the andromonoecious, the change involving only the mutation of a single gene. It is of some interest that this change would involve a dominant mutation. Moreover, it has been shown in this paper, that there is some tendency for the production of occasional pistillate flowers on hermaphroditic plants, though the reverse has never been observed. This change in sex form, however, appears not to be hereditary in the cases which have been studied by the writer.

ASSOCIATION OF FRUIT SHAPE WITH SEX FORM OF THE FLOWER

In examining the flowers of F_2 and back-cross progenies, it was noticed that pistillate flowers usually had a long ovary, while hermaphroditic flowers had shorter ovaries, tending more nearly to the round form. When the fruit came to maturity, the same general relation in fruit shape was observed. This observation also applies in general to the parental varieties of melons, cucumbers and watermelons. In two of the melon crosses the individual fruits from F_2 plants were measured. Dividing the equatorial by the polar diameter gives an index for fruit shape, $\frac{ED}{PD}$, which is less than 1 for oblong fruits and more than 1 for oblate fruits. In cross 8 the factor for fruit shape was based on measurement of five or more fruits from each plant; in cross 13 only one representative fruit of each plant was measured. Table 5 gives the average of the fruit shape ratios for the pistillate and hermaphroditic plants in these crosses.

TABLE 5

AVERAGE FRUIT SHAPE RATIOS, $\frac{ED}{PD}$, FOR PISTILLATE- AND HERMAPHRODITIC-FLOWERED PLANTS IN THE F_2 GENERATION OF MELON CROSSES

Cross	Pistillate-flowered plants		Hermaphroditic-flowered plants	
	Number of plants	Average of shape ratios	Number of plants	Average of shape ratios
8. Salmon Tint x Freiland............	53	.628±.005	14	.783±.014
13. Snake x Salmon Tint....	69	.291±.005	26	.371±.008

The shape ratios for the parental varieties used in these crosses were as follows: Salmon Tint, .941; Freiland, .625; and Snake, .105. It is clear from table 5 that the fruit shape differs in the F_2 phenotypes, the fruits developing from hermaphroditic flowers being the thicker in proportion to their length. The difference between the means of the shape ratios for the two types is about 10 times its probable error in both crosses. It cannot be said with certainty that this fact is due to linkage of a genetic factor for fruit shape with the factor for sex-expression. The association may be due to

the action of a single factor, one character being the result of the other. However, the association of these characters was the same in the F_2 phenotypes as it was in the parental varieties. It may be recalled, also, that fruit arising from the occasional pistillate flowers in varieties normally hermaphroditic-flowered, show a similar deviation in fruit shape from the normal for the variety.

CARPEL NUMBER IN THE FLOWERS AND THE FRUITS

The members of the genus *Cucumis* are generally considered to be trimerous both as to the gynoecium and the androecium. Naudin[7] considered the cucumber, *C. sativus*, to represent the basic condition for the androecium of the Cucurbitaceae—two stamens each with two thecae, and one with a single theca. The same arrangement is found also in most varieties of *Cucumis melo* and *Citrullus vulgaris*. This condition has been referred to by some writers as "two and a half" stamens. It is usually accompanied by a tri-carpellate ovary surmounted by a three-lobed stigma. Vuillemin,[13] however, argues that both androecium and gynoecium were originally hexamerous, each consisting of two trimerous whorls; by abortion of one member of one whorl, these organs became pentamerous, and by the suppression of one entire whorl, trimerous. Heimlich,[3] in his study of the staminate flower in cucumber, could find no evidence of aborted vascular bundles supposed to supply missing stamens, and because of this, concluded that the double stamens are the complete normal type, while the single stamen is a half or reduced form. Then the androecium would be trimerous, as the gynoecium certainly appears to be, in the cucumber.

The writer found two varieties of melons, the Golden Beauty Casaba (*Cucumis melo* var. *inordorus* Naud.) and Pomegranate (*C. dudaim* L. or *C. melo* var. *odoratissimus* Naud.) which are pentamerous both as to androecium and gynoecium. There are five single stamens (like the half stamen of other *Cucumi*) in both the staminate and hermaphroditic flowers, and there are five separate locules in the ovarian cavity. These characteristics have remained constant in inbred lines of the Casaba, to the fourth inbred generation. A cross-section of the Casaba fruit showing the five carpels is presented in figure 3, and one of a cantaloupe melon showing the ordinary tri-carpellate condition, in figure 4.

It has also been observed that all varieties of melons, except those that are perfectly smooth on the surface, like Honey Dew, or those in which the surface is broken by numerous fine corrugations, as in Snake, show ten distinct lobes to the fruit, with longitudinal sutures between them. This is illustrated in extreme degree in figure 5. These lobes correspond to the ten parts of the calyx and corolla, two alternating whorls of five parts each. The outer part of the melon fruit then consists of receptacle fused with and enclosing the pericarp. It seems to the writer that the flowers of *Cucumis* and of *Citrullus* were originally pentamerous throughout. This stage of evolutionary

Fig. 3. Cross sections of fruit from two inbred lines of Casaba melon, showing the five carpels. The inked lines on the fruit at the right indicate the division between the carpels.

development is represented today by the two varieties mentioned above. But most present-day varieties of melons present a trimerous gynoecium. In the ovary, two carpels have been eliminated, either by union or by abortion, resulting in the tri-carpellate condition, now found in most melons, all cucumbers and nearly always in watermelons. Both cantaloupe melons and watermelons occasionally produce fruits with four carpels, however. This is most common in watermelons in varieties which have hermaphroditic flowers and which therefore may be presumed to be nearer the primitive type. But the evolution of the androecium has not advanced so far, and now generally presents a pseudo-trimerous condition, with one single and two double stamens.
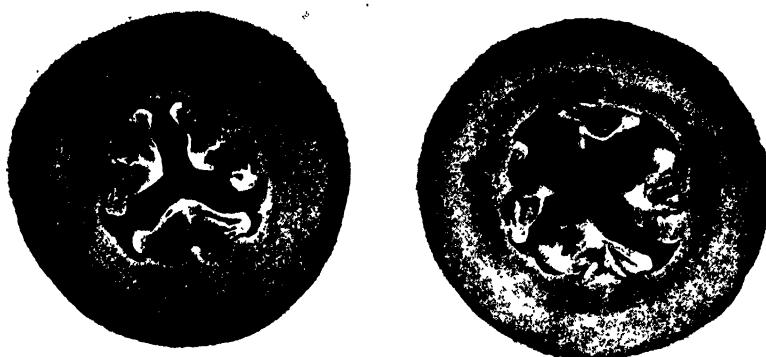
Fig. 4. Cross sections of fruit of the Salmon Tint melon. The one on the left shows the usual tri-carpellate condition found in this variety. At the right is one of the four-carpellate fruits which occasionally occurs.
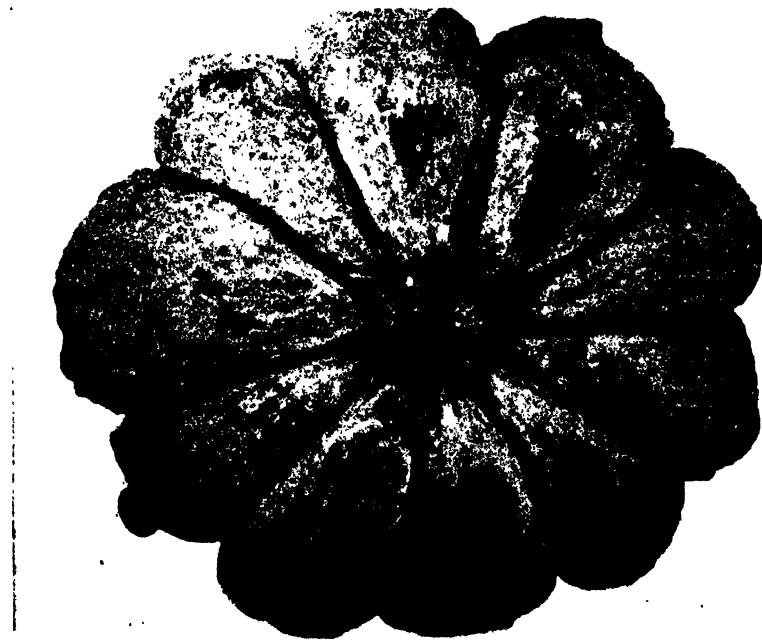
Fig. 5. A cantaloupe melon viewed from the stem end. It shows the ten lobes of the fused receptacle, separated by deep sutures. Most other varieties of *Cucumis melo* show the same condition, though the lobes are usually not so prominent.

INHERITANCE OF CARPEL NUMBER

The foregoing discussion has shown the evolutionary trend by which tri-carpellate varieties are assumed to have arisen from a primitive five-carpellate form. With the view of determining the genetic basis for the distinction between these two forms, crosses were made between the pentamerous Casaba and the tri-carpellate Salmon Tint, Persian and Hoodoo varieties. In these crosses, as well as several others which will not be discussed here, the tri-carpellate form was completely dominant in the F_1 generation, as was also the pairing of four of the stamens, the pseudo-trimerous androecium. The results in the F_2 populations are given in table 6.

TABLE 6

DISTRIBUTION OF CARPEL NUMBER IN THE F_2 GENERATION OF CROSSES BETWEEN TRI-CARPELLATE AND FIVE-CARPELLATE VARIETIES OF *Cucumis melo*

Cross	Plants with tri-carpellate fruit	Plants with five-carpellate fruit
Casaba x Hoodoo.........	35	14
Casaba x Salmon Tint	27	10
Salmon Tint x Casaba.....	25	14
Casaba x Persian.....	24	11
Total...............	**111**	**49**
Calculated 3 : 1 ratio	*120*	*40*

Deviation $= 9 \pm 3.69$: $\dfrac{D}{E} = 2.4$; *probability per* $100 = 10.55$.

Although this is not as close an approximation to the theoretical 3 : 1 ratio as was obtained in the crosses involving sexual forms, yet the fact that such a deviation as the one occurring here may be expected to occur once in every ten trials under the laws of chance, justifies the inference that in this case also a single gene difference is involved. Further tests, however, are desirable.

Definite segregation for carpel number occurred in F_2, though there were some intermediate forms. Thus some of the plants classified as five-carpellate in table 6, had a few fruit with only four carpels. And many of the plants classified as having tri-carpellate fruit bore some with four carpels. Figure 6 shows a five-carpellate fruit of an F_2 plant of the cross Casaba × Persian, in which is observed

a tendency of four placentae to unite in pairs, leaving the fifth free. The number of stamens in the F_2 plants was found generally to agree with the number of carpels.

Seed were taken from three tri-carpellate and from three five-carpellate F_2 plants of the Casaba × Hoodoo cross. Two of the former and all of the latter, bred true, while one of the tri-carpellate segregated again in the F_3 generation.

Fig. 6. Cross section of five-carpellate fruit from an F_2 segregate of the cross Casaba × Persian, showing a tendency of four carpels to pair in two's.

Jones and Raynor,[5] studying carpel number in crosses between bi- and tri-carpellate varieties of *Bryonia*, found more complicated conditions to exist. They assumed, however, that there were two factors, G_1 and G_2, for the bi-carpellate ovary, the constitution of the tri-carpellate being $g_1 g_2$. The evidence on the behavior of this character in melons indicates that in this plant, probably only one genetic factor is involved in determining carpel number. It is interesting, moreover, that the five-carpellate form, which seems to be the most primitive from the phylogenetic point of view, is recessive. In this case, as in the study of sex forms, evolution by dominant mutations is suggested.

Association of Fruit Shape with Carpel Number

The fruits from some F_2 progenies of the melon crosses were measured and the average ratio for fruit shape was calculated, using five or more fruits from each plant. The results are given in table 7.

TABLE 7

Average Fruit Shape Ratios $\dfrac{ED}{PD}$ for Plants Bearing Mostly Tri-Carpellate and Mostly Five-Carpellate Fruits

Cross	Tri-carpellate plants		Five-carpellate plants	
	Number of plants	Average of fruit shape ratios	Number of plants	Average of fruit shape ratios
Casaba x Salmon Tint... ...	27	.924±.013	10	1.021±.019
Salmon Tint x Casaba	23	.938±.015	14	1.020±.014
Casaba x Persian.	23	.904±.011	11	1.027±.016

It is seen that there is a definite association between carpel number of the fruits, and their shape. The five-carpellate phenotypes have fruits that are in general round or slighly oblate. The tri-carpellate fruits are oblong or oval. The difference in mean fruit-shape ratio is four to six times the probable error. The relation found in the Casaba × Persian cross is the opposite of that existing in the shape of the parental varieties. Carpel number, therefore, will require consideration in genetics studies on fruit shape. Similar results, both as to the dominance of low carpel number, and the association of carpel number and shape, have been obtained by the writer with tomato fruits.

SUMMARY AND CONCLUSIONS

It is shown that the cultivated varieties of *Cucumis melo* are preponderantly andromonoecious, relatively few varieties being monoecious. The opposite distribution exists in the varieties of *Cucumis sativus* and *Citrullus vulgaris*.

The varieties of these three species were found to be constant in the sex arrangements of the carpellate flowers, except that in a number of andromonoecious varieties of *Cucumis melo*, about one flower in 186 was transformed to the pistillate condition. The association of

elongated fruit shape with sex of pistillate flowers, permits of esti-mating that this "sex reversal" occurred in about one in 400 flowers that produced fruit, in inbred lines of the Salmon Tint variety.

The results of crossing monoecious × andromonoecious varieties, and *vice versa,* indicate that the monoecious condition depends upon a single dominant factor in all three species. Very close approxima-tion to a ratio of 3 monoecious to 1 andromonoecious plants was obtained in F_2, with a 1:1 ratio in the backcrosses to the recessive parental variety.

It is suggested that hermaphroditic flowers are the more primitive type from the evolutionary point of view, and that the pistillate form has arisen from them by dominant gene mutation.

The flowers of *Cucumis* and *Citrullus* are considered to have been originally pentamerous throughout. The tri-carpellate ovary, which is now the prevailing type in most varieties, probably arose from the five-carpellate form, two carpels having been eliminated. In the evolution of the androecium, the pentamerous condition as now repre-sented in Casaba melons, with five single stamens, preceded the pseudo-trimerous condition (two double and one single stamen), which accompanies the tri-carpellate ovary.

Crosses of tri-carpellate with five-carpellate varieties show that the latter, probably the more primitive form, is recessive, and the segre-gation in F_2 can probably be explained on a single major factor difference. The reduction of carpel number, with corresponding fusion of stamens, suggests evolution by means of dominant mutations.

An association of sex conditions, in the carpellate flowers, with shape of the resultant fruit, was found. In F_2 progenies, fruits from hermaphroditic-flowered plants were more nearly globose than those of pistillate-flowered plants. A similar association of shape with carpel number was observed, five-carpellate fruit of F_2 phenotypes being globose or oblate, while tri-carpellate were oval or oblong.

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TRANSMISSION OF TOMATO YELLOWS, OR CURLY TOP OF THE SUGAR BEET, BY EUTETTIX TENELLUS (BAKER)

HENRY H. P. SEVERIN[1]

(Contribution from the Division of Entomology and Parasitology, College of Agriculture, University of California, cooperating with the United States Department of Agriculture, Bureau of Plant Industry).

INTRODUCTION

According to the literature, the cause of tomato yellows (western yellow blight) has remained a mystery since 1906. It was not until 1927 that the beet leafhopper, *Eutettix tenellus* (Baker), which transmits curly top to sugar beets, was associated as a carrier of the same disease to tomatoes.

Carsner and Stahl (1924)[2] reported tomato as susceptible to curly top, but from a study of the symptoms in the greenhouse, they state that "this disease has not been found in commercial plantings."

McKay and Dykstra (1927) came to the conclusion, on circumstantial evidence, that tomato yellows is caused by the virus of sugar-beet curly top.

Shapovalov (1927a) proved conclusively that curly-top virus, when introduced into the tomato plant by means of infective beet leafhoppers, produced typical symptoms of yellows with tomatoes grown out-of-doors.

During 1927, experiments conducted on the University Farm at Davis, in cooperation with J. T. Rosa and M. Shapovalov, demonstrated

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[2] Complete data on papers cited will be found in "Literature Cited," pages 270–271, arranged by author and date.

beyond doubt that when infective beet leafhoppers, *Eutettix tenellus* (Baker) were transferred from curly-top beets to healthy tomato plants, typical symptoms of tomato yellows developed.

All of the foregoing conclusions were based on a comparison of the symptoms of tomatoes inoculated with curly top by the beet leafhopper in the greenhouse or out-of-doors, with symptoms of plants naturally infected with tomato yellows. The transfer of curly top from tomatoes experimentally inoculated by infective beet leafhoppers in the field and showing typical symptoms, and from naturally infected tomatoes, back to beets has not been demonstrated. The chief object of the experiments reported in this paper is the demonstration of this transfer.

Other aspects of this subject discussed in the paper are the injury to tomatoes by this disease in natural and migratory breeding areas of the beet leafhopper, the varieties naturally and experimentally infected with curly top, the symptoms in the greenhouse and field, the longevity of the insects on tomato plants, the infection of a tomato plant with two virus diseases, and the relation of the spring migrations of the pest to the time of transplanting tomatoes.

NAMES OF THE DISEASE

The name of this disease first appeared in the literature as 'summer blight' by Smith (1906), Smith and Smith (1911), Rogers (1916), Rosa (1923); then 'yellow blight' by Huntley (1902), Henderson (1905), and Humphrey (1914); next 'western blight' by Thornberg (1912), Eastham (1920), Yaw (1924); and finally 'western yellow blight' by McKay (1921), McKay and Dykstra (1927), Heald (1922), Hungerford (1923), Shapovalov (1925, 1925*a* and 1927), Lesley (1926), Rosa (1927), and Severin (1927). The word 'tomato' has been omitted in some of the above names of this disease, or the list would be longer.

Shapovalov (1925*a*) suggested "that the name 'western yellow tomato blight' be changed to 'tomato yellows'," and this change was approved by the Pacific Division of the American Phytopathological Society (Shapovalov, 1927), but has not been acted upon by the committee on nomenclature. For the present purposes, and to avoid confusion in the Experiment Station literature, that name has been used for the disease in this paper, although it has been most commonly known as western yellow tomato blight.[3]

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[3] The name 'tomato curly top' has been proposed to the Committee on Nomenclature of the American Phytopathological Society, but no action has been taken as yet.

Injury in Natural and Migratory Breeding Areas of Beet Leafhopper

It has been known in California for a long time that outbreaks of curly top of sugar beets and tomato yellows show some correlation. During 1905 a disastrous outbreak of beet curly top occurred, and tomato yellows, according to Smith (1906), was more general than ever before, completely ruining many fields in southern California and almost all in the San Joaquin Valley. During 1919 and 1925, curly top destroyed most of the late plantings of sugar beets and seriously reduced the tonnage of early plantings in the San Joaquin and Sacramento valleys and the interior regions of the Salinas Valley; in the same years tomato yellows destroyed most of the tomato crop in these valleys. The disease of these two crops is subject to regional variations, being more severe in or near the natural breeding areas of the beet leafhopper in the San Joaquin and Salinas valleys than in the coastal or other migratory districts.

In years between outbreaks of the pest, the severity of the disease varies according to the number of leafhoppers which invade the cultivated regions in different parts of a natural breeding area. The plains and foothills of most of Kern County, in the southern part of the San Joaquin Valley, are natural breeding grounds, except the Sierra Nevada foothills near the northern end of the county. With this enormous breeding area, even with a relatively low population of the insects, a profitable crop of tomatoes can rarely be grown in Kern County. The foothill breeding regions of the northern San Joaquin Valley are not as favorable as the middle and southern portions of the valley, and yellows is less prevalent, especially in tomato fields planted after the spring dispersal of leafhoppers ceases.

The direction of the wind at the time that the large flights occur is also a factor controlling the amount of tomato yellows in migratory regions of this insect. During 1927 large numbers of beet leafhoppers migrated from the San Joaquin Valley into the fog belt, and about five per cent of the tomatoes were blighted in the districts east of the region between San Francisco and Monterey Bay, as compared with less than one per cent in the Sacramento Valley, where fewer leafhoppers migrated. At the time that the maximum flights occurred in the middle San Joaquin Valley on May 4, the spring brood adults flew with an easterly wind over the Coast Range into the fog belt. Migratory flights into the Sacramento Valley are probably associated with south or southeasterly winds blowing from the San Joaquin Valley and calm spells in the vicinity of Suisun Bay.

Varieties of Tomatoes Naturally and Experimentally Infected with Curly Top

All canning and shipping varieties of tomatoes grown in California are naturally infected with tomato yellows. The following varieties have been experimentally infected with the disease: Alameda Trophy, Earliana, First Early, Globe, King of the Earlies, San Jose Canner, Santa Clara Canner, Special Early, Stone, and Wild Mexican. Lesley (1926) reports that varieties of the dwarf type, Red Pear, and certain selected lines of the Canner type, possess a moderate degree of resistance.

Symptomatology

In the Field.—The principal symptoms of tomato yellows which develop in the field are an inward rolling of the leaflets along the mid-rib (fig. 2); the petiole and mid-rib frequently curve downward (fig. 1), giving the leaf a drooping but not wilting appearance; and the leaves become somewhat thickened and crisp. Later the leaves assume a yellow color with purple veins. The purpling of the veins cannot be considered a reliable symptom of blight, since healthy plants may show purple venation, especially during late summer and autumn. The stems become hollow through the drying of the pith. With the first appearance of these foliage symptoms, the plant stops growing and assumes an erect or rigid habit. Rosa (1927) states that the foliage symptoms are probably due to the abnormal accumulation of carbohydrates in the affected plants, which in turn results from the stoppage of vegetative growth. If small fruits have been formed, they ripen prematurely and the seeds are abortive. A decay of the roots occurs, usually beginning at the tips of the smaller roots. The plant finally dies, the leaves and stems turning brown (fig. 1).

All the field symptoms of yellows develop in the fog belt, but the incubation period of the disease may be longer than in the interior regions.

Tomato seedlings grown and transplanted on the University Farm at Davis were inoculated with curly top of beets at different stages of growth by means of infective leafhoppers, and a study of the symptoms was made during the season. Tomatoes inoculated with the disease two days before transplanting in the spring either developed mild symptoms of yellows or turned yellow and died. Non-infective beet leafhoppers, however, after feeding on the plants that were yellow but had no other symptoms, were transferred to sugar beets,

and symptoms of curly top developed (table 2). Tomatoes infected with curly top three days after transplanting in the field developed typical symptoms of yellows (fig. 1). All tomatoes infected before or shortly after transplanting died before reaching the flowering stage.

Fig. 1. Left, tomato naturally infected with tomato yellows shortly after transplanting, showing stunted plant with downward bending petioles and leaves and decayed root. Right, dried tomato plant which died as a result of tomato yellows.

Old plants inoculated with curly top, with few exceptions, seemed to withstand the disease. Tomatoes transplanted on May 14 were inoculated with curly top by about 100 infective nymphs to a plant on August 15, or about three months after transplanting; they continued normal growth, but some developed a striking sulphur-yellow discoloration of the foliage (fig. 3) on the terminal shoots, with no

other symptoms, by October 19. An examination of these plants on November 11 showed that nine of seventeen plants developed the rolled leaves, a deep purpling of the whole leaf surface as well as the veins, in addition to the conspicuous yellowing of the terminal shoots. The leaflets on some of the plants were dwarfed (fig. 4) near the tips. These late-inoculated plants produced fruit approaching normal in size.

In walking between the rows of tomatoes, diseased plants are easily recognized from a short distance by a lag in growth. The disease may come on gradually or rather suddenly in the field, and affected plants are not uniformly distributed. Later in the season tomato fields often appear spotted with dead brown or missing plants.

In the Greenhouse.—After the curly-top virus was introduced into tomato plants by means of infective beet leafhoppers, a study was undertaken of the symptoms which developed in the greenhouse. In the first experiment the tomatoes were grown in ten-inch pots in the greenhouse and each plant was enclosed in a large cage covered with lawn. The first reliable symptom of curly top to appear in the common California commercial varieties of tomatoes in the greenhouse is transparent venation. An inward curl of some of the leaflets occurs (fig. 5), especially in older plants. Purple venation often does not develop with infected tomatoes enclosed in cages. White excrescences (pl. 1, fig. 1) sometimes appear on the veins resembling somewhat the wart-like protuberances on curly top beets. A yellowing often develops between the veins, while the veins remain green (pl. 1, fig. 2). A marked stunting of young tomato plants occurs (fig. 6). Later the entire plant turns yellow and dies.

In previous years these same symptoms developed in cages and the tomato was considered to be susceptible to curly top. Plant pathologists who examined these infected tomatoes came to the conclusion that these symptoms were not those of tomato yellows as it develops in the field.

In view of the fact that tomatoes enclosed in cages under high temperatures in the greenhouse assume a spindling habit, another experiment was undertaken. Tomatoes grown out-doors were transplanted into soil in the floor of the greenhouse. The lower and upper vents and large windows in the front end of the greenhouse were kept open day and night to allow circulation of air. The tomatoes were inoculated by infective nymphs which were dropped on each plant with a pipette. The symptoms of yellows taken individually appeared in mild form, but the totality or complex of field symptoms such as occur in the hot interior regions of California failed to develop.

Fig. 2.   Leaf from tomato plant naturally infected with tomato yellows showing inward-rolled leaflets.

Fig. 3.   Leaf from tomato plant infected with curly top on August 15, showing white leaflets which were sulfur yellow in October and November.

The reliable symptom of curly top, the cleared veinlets, were again discernible on the younger leaves. Plant pathologists who examined these diseased tomatoes all agreed that the symptoms resembled a mild form of tomato yellows.

McKay and Dykstra (1927) infected tomato plants with the beet leafhoppers and "typical symptoms of western yellow blight developed in the greenhouse. These were general yellowing of the foliage, a rolling of the leaves, a purpling of the veins, and a marked stunting of the plant." McKay and Dykstra failed to mention the development of transparent venation under greenhouse conditions.

A comparison of the symptoms which developed in the field with those in the greenhouse indicates that transparent venation and the white excrescences on the veins must be considered greenhouse, and not field symptoms. A large number of small, stunted, diseased tomato plants were examined on June 16, 1925, on the ranch of the California Packing Corporation near Rio Vista in the Sacramento Valley, but transparent venation was absent. This reliable symptom of curly top has not been found in older diseased tomato plants in the San Joaquin and Salinas valleys during 1926. Tomato plants infected with curly top by the beet leafhopper in different stages of growth on the University Farm at Davis during 1927 did not show the cleared veinlets.

Incubation Period.—The incubation period varies from 2 weeks to 4 weeks. In 1927 an unusually cool spring prevailed, no very high temperatures occurring until June 15. From June 13 to June 15, the maximum temperatures were 96°, 99°, and 103° Fahrenheit. Tomatoes inoculated May 14–16 showed symptoms of yellows on June 10, an incubation period of 24 to 26 days. Plants inoculated May 30 developed symptoms of yellows on June 15, an incubation period of 16 days. These experiments indicate that although tomato yellows may develop before the first 'hot spell,' high temperatures and perhaps other factors materially shorten the incubation period.

LONGEVITY AND LIFE HISTORY OF BEET LEAFHOPPERS ON TOMATO PLANTS

Experiments were made to determine the longevity of the last living male and female beet leafhoppers of the spring, summer, and winter broods, when these were confined on tomato plants. These experiments were often repeated with different lots of adults. The results are shown in table 1.

Fig. 4.   Terminal shoot of tomato affected with curly top, showing dwarfed leaflets.

Fig. 5.   Leaves showing inward curl of leaflets from a plant infected with curly top in the greenhouse.

TABLE 1

Longevity of Last Living Male and Female Beet Leafhopper on San Jose
Canner Tomato

Brood	Longevity of males *days*	Temperatures			Longevity of females *days*	Temperatures			Height of tomato plants *inches*
		Mean maximum °F	Mean minimum °F	Mean °F		Mean maximum °F	Mean minimum °F	Mean °F	
Spring........	3–4	111.7	60.2	85.9	4–9	111.5	60.0	86 6	6–8
Spring	7–8	91.0	66.2	77.8	16–19	92.8	66 0	79 4	14–24
Summer...	6.	107.8	66.7	87.2	13	109.6	64 8	87.2	6–8
Summer....	14–23	102.8	61.2	82.0	28	101.5	61 5	81.5	18–24
Winter........	7	82.0	62.2	72.1	22	81.2	63.4	72.3	6–8, 6–19
Winter........	5–9	78.9	61.2	70.0	23	75.2	56 6	65 9	8–19, 19–25

It is evident from table 1 that the males live for a shorter period than the females when confined on a tomato diet. The adult life of the spring and summer broods on tomatoes at transplanting size (6 to 8 inches) is shorter than on older tomatoes (14 to 24 inches). The males of the winter brood die during the winter and many of the specimens tested for longevity may have been near the end of their natural life. The experiments made on the overwintering females show very little difference in the adult life on transplanted and older tomatoes.

The beet leafhopper failed to complete its life cycle on California canning and shipping tomatoes listed under experimentally infected varieties.

Curly-top Inoculation of Tomatoes in the Field

Tomatoes grown on the University Farm at Davis were inoculated with the curly-top virus by means of infective beet leafhoppers. In a series of seven experiments, inoculations were made in tomatoes at different stages of growth during a period of three months. In order to carry on these experiments, four rows of tomatoes were transplanted on May 14 to 17, each row containing from 35 to 40 plants (fig. 7). The variety used was a selection of San Jose Canner. The soil was kept free from host plants of the beet leafhopper and weeds susceptible to curly top.

Experiment 1.—In the first experiment 40 healthy tomato plants were inoculated with curly top in the greenhouse, using 10 infective male beet leafhoppers for a period of two days on each plant. Males were used rather than females, to prevent oviposition. These tomatoes

were transplanted on the University Farm on May 17. The 40 infected plants either developed symptoms of tomato yellows or turned yellow and died (table 2).

Fig. 6. Comparison of growth of two tomatoes planted on the same date: left, inoculated with curly top by infective beet leafhoppers; right, check or control plant on which non-infective males fed.

Fig. 7. Plot used in experiments 1 to 7. Row 1 was used in experiments 2 and 3, and shows the badly diseased plants in the foreground. One plant shown in the background remained healthy, all others died. Rows 2 and 4 were used as a check at the time that this photograph was taken, but later the tomato plants were infected in experiments 4 to 7. Row 3 in experiment 1, the tomato seedlings were infected with curly top before transplanting and all of the plants died.

Experiment 2.—In the second experiment, repeated eight times, a curly-top beet was planted near a healthy tomato plant, and both were covered with a cylindrical cage in which 25 infective males were liberated. The beet leafhoppers did not all congregate on the beets—a few were actually seen feeding on the tomato plants. The cages were removed 13 days later and a few males were still alive in each cage. The eight tomato plants developed typical symptoms of yellows, as indicated in table 2.

Experiment 3.—In the third experiment 20 healthy tomato plants were enclosed in 20 cages in each of which 10 infective males were set free. The cages were removed at the end of one week. Table 2 shows that all but one tomato plant developed symptoms of yellows. Terminal shoots were repeatedly removed from this plant during the season, but non-infective males which had fed on them failed' to transmit curly top to sugar beets. It is evident that 10 infective beet leafhoppers confined in a cage for a week did not infect this tomato plant. The number of bugs required for 100-per-cent infection should be taken into consideration in the development of a tomato resistant to curly top.

Experiments 4 to 6.—In the next three experiments 10 or 20 infective nymphs or males were confined in small leaf-cages (fig. 8), but in fastening the cages to the leaves some of the hoppers escaped. Two of 140 tomato plants to be inoculated during the season were either naturally infected or were inoculated with the disease by bugs which escaped while attaching the leaf-cages. A high mortality of the insects occurs in leaf-cages during hot weather. These cages were often torn from the plants by heavy winds. Table 2 indicates that from 33.3 to 61.5 per cent of the plants thus treated developed symptoms of the disease.

TABLE 2

CURLY-TOP INOCULATION OF TOMATO PLANTS AT VARIOUS STAGES OF GROWTH UNDER FIELD CONDITIONS

Experiment No.	Dates plants were inoculated	Type of cage	Number of beet leaf-hoppers on each plant	Date cages were removed	Number of plants inoculated	Number of diseased plants	Percentage of diseased plants
1	May 14–16.......	Cylindrical....	10 males........	May 17...	40	40	100.0
2	May 17–30.....	Cylindrical.....	25 males........	May 30......	8	8	100.0
3	May 30–June 6..	Cylindrical.....	10 males.......	June 6.	20	19	95.0
4	June 6–27........	1 leaf-cage.......	10 nymphs ..	June 27.....	26	16	61.5
5	June 27–July 25	1 leaf-cage......	10 males.........	July 25......	12	6	50.0
6	July 25–Aug. 15	2 leaf-cages......	20 males.........	Aug. 15......	15	5	33.3
7	Aug. 15............	No cage	100 nymphs .	....	17	9	52.9
					138	103	74.6

Experiment 7.—In the last experiment 100 infective nymphs were dropped on the inner leaves of 17 large tomato plants on August 15. The last examination of these plants on November 11 showed that nine plants had developed symptoms of yellows and eight were *apparently* healthy, as indicated in table 2.

Fig. 8.   Leaf-cages attached to tomato leaves.

Check or Control.—Two thousand tomato plants used as a check or control, about 500 feet north of the four rows of inoculated plants mentioned above, showed less than 1 per cent of yellows at the end of the season.   These tomatoes were transplanted on May 14 to 16,

and included a selection of San Jose Canner. Sugar beets showing 100 per cent curly top were growing about 50 feet northwest from this tomato field used as a check.

Curly-top Transmission from Inoculated Tomatoes to Sugar Beets. Cross inoculations were made from the tomato plants showing symptoms of yellows in experiments 1 to 7, to healthy sugar beets. Non-infective males after feeding from 2 to 6 days on small diseased plants or on several terminal shoots removed from large diseased plants were transferred to healthy sugar-beet seedlings. The results are given in table 3.

TABLE 3

CURLY TOP TRANSMITTED TO SUGAR BEETS FROM TOMATOES EXPERIMENTALLY
INOCULATED BY INFECTIVE BEET LEAFHOPPERS

Date of inoculating tomatoes	Dates non-infective males fed on inoculated tomatoes	Number of non-infective males on each inoculated tomato	Number of inoculated tomatoes tested	Number of beets inoculated	Number of beets infected	Percentage of beets infected with curly top
May 14–16............	June 30–July 1....	10	16	16	15	93.7
May 17–30...	June 30–July 1....	25	8	8	8	100.0
May 30–June 6....	June 30–July 1....	10	10	10	10	100.0
June 6..................	Aug. 15–18..........	20	6	6	6	100.0
June 27................	Aug. 15–18..........	20	6	6	6	100.0
July 25................	Nov. 12–17..........	15	6	6	5	83.3
Aug. 15................	Oct. 20–23..........	15	9	9	7	77.7
Aug. 15........	Nov. 11–17..........	15	11	11	9	81.8

Table 3 shows that curly top was transmitted to sugar beets from infected tomato plants as follows: spring 93.7 to 100 per cent; summer 100 per cent; and autumn 77.7 to 83.3 per cent. The size and age of the spring, summer, and autumn plants are not comparable.

Seventeen tomato plants, each of which were inoculated with 100 infective nymphs on August 15, in experiment 7, were tested on October 20 to 23 and November 11 to 17, for curly-top transmission to sugar beets. A comparison of the results obtained in each test may be illustrated as follows:

```
                             C  C     C  C  C     C    ?  ?  C
Oct. 20–23.   Plant No...............  {  17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
                             H  H  H        H           H     H           H  H

                             H  ?  H        H                 H     ?     H  H
Nov. 11–17.   Plant No...............  {  17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
                                C  C     C  C  C  C  C     C     C
```

C indicates that curly top was transmitted to sugar beets from the inoculated plants.
H indicates that the plant was apparently healthy.

Plant No. 18 was apparently healthy in October; in November this plant showed mild symptoms of yellows, but curly top was not transmitted to sugar beets. Plant No. 26 was apparently healthy in October, but in November the terminal shoots were yellow, with no other symptoms. Curly top was communicated from this plant to beets. Plants 29 and 30 showed a yellowing of leaves on the tips of the stems in October and a mild form of yellows in November. Cross inoculation of curly top from plant No. 29 was made to beets, but negative results were obtained from plant No. 30.

CURLY-TOP TRANSMISSION FROM TOMATOES NATURALLY INFECTED WITH
YELLOWS TO SUGAR BEETS

In 1925 and 1926 it was demonstrated that during the autumn non-infective beet leafhoppers do not always transmit curly top from tomatoes naturally infected with yellows to sugar beets. During 1927 three shipments of tomato plants naturally infected with yellows, grown on the grounds of the United States Cotton Field Station at Shafter in the southern San Joaquin Valley, were received from M. Shapovalov and F. S. Beecher. Non-infective males were fed on the diseased plants for a period of two days and were then transferred to healthy beet seedlings. Table 4 shows the number and percentage of beets which developed curly top.

TABLE 4

CURLY TOP TRANSMITTED FROM TOMATOES NATURALLY INFECTED WITH YELLOWS
TO SUGAR BEETS

Number of tomatoes naturally infected with yellows	Dates non-infective males fed on diseased tomatoes	Number of non-infective males on each diseased tomato	Number of beets inoculated	Number of beets infected with curly top	Percentage of beets infected with curly top
12	May 28–30	15	12	12	100.0
15	June 28–30	15	15	14	93.3
49	Aug. 24–26	15	49	35	71.4

It is evident from table 4 that the beet leafhoppers transmitted curly top from 100 per cent of the tomato plants naturally infected with yellows to sugar beets during the spring. During the summer, curly-top transmission from diseased tomatoes to sugar beets varied from 71.4 to 93.3 per cent. The summer plants were larger and older than those used in the spring.

TOMATO PLANTS NATURALLY INFECTED WITH BOTH TOMATO YELLOWS
AND MOSAIC

During 1926, 100 acres of tomatoes growing on the Spreckels ranch
near King City in the Salinas Valley were destroyed by yellows,
mosaic, and possibly other diseases. Eighty acres of sugar beets
affected with curly top had been plowed under on this ranch, causing
a dissemination of the beet leafhoppers to other cultivated plants and
weeds. The terminal shoots from many tomato plants showing only
symptoms of mosaic were removed, and the cut ends were placed in
tumblers filled with water while the tips projected into cages. Non-
infective males after feeding on the shoots affected with mosaic trans-
mitted curly top to sugar beets. This transmission to beets demon-
strated that the mosaic tomatoes were also naturally infected with
the virus of curly top, but the symptoms of the latter had not
developed. An infection of mosaic and curly top in the same beet is
common in the beet fields of California, but the leaves always show
symptoms of both diseases.

An experiment was conducted in the greenhouse to determine
whether symptoms of curly top and mosaic develop with tomatoes
infected with the virus of the two diseases. Tomatoes were inoculated
with mosaic on May 20 by crushing and rubbing the diseased leaves
on healthy ones. Ten infective beet leafhoppers were fed on each
plant inoculated with mosaic on May 20 to 22. The tomatoes inocu-
lated with the two diseases were removed from the cages and trans-
planted into soil in the floor of the greenhouse. The cleared veinlets
of curly top developed in all of the inoculated plants but were diffi-
cult to distinguish from normal venation in mosaic leaves. In some
cases the leaflets nearest the terminal end of the leaf showed pro-
nounced transparent venation while the basal leaflets or those nearest
the petiole showed mosaic symptoms (pl. 2, fig. 1). In addition to the
cleared veinlets of curly top, other symptoms developed on these
plants as follows:

1. Mosaic, no yellows (pl. 2, fig. 2).

2. Mosaic on the leaves of the terminal shoots and a mild form of
yellows on the leaves of the lower portion of the plant.

3. Mosaic on the younger leaves and a mild form of yellows affect-
ing the entire plant.

Non-infective beet leafhoppers were fed July 2 to 4 on the tomatoes
infected with the two diseases, with symptoms described above (1, 2,
3), and then were transferred to healthy sugar beets, which developed
curly top. This experiment should be repeated under field conditions.

RELATION OF SPRING MIGRATIONS TO TIME OF TRANSPLANTING TOMATOES

Flights.—According to E. A. Schwing, a small flight of the beet leafhoppers into the beet fields of Sacramento Valley occurred on April 24, 1927. The maximum flights occurred on May 4, in the Delta regions, and on May 12, in the Sutter Basin and Marysville districts. No further increase of the leafhoppers took place in the beet fields after May 12.

After a large flight occurs the adults are generally distributed on all green vegetation. The first stimulus after a flight is apparently a food stimulus, and the insects are often found on unsuitable food plants on which they cannot survive. Later, however, the hoppers congregate on their most favorable food and breeding plants.

Occurrence of Beet Leafhoppers on Tomatoes.—During the spring and summer of 1927, an effort was made to find the beet leafhopper on the 2,000 tomato plants used as a check or control at Davis. The plants were shaken and insects were captured on the soil beneath with a pipette. During the season not a single specimen of *Eutettix tenellus* was taken on tomatoes. The first examination was made on May 17, and not immediately after the large flight on May 4. In a nearby beet field the pest was generally abundant. The percentage of curly top which appeared in the beet field early in the season was as follows: May 17, 20 per cent; May 30, 27 per cent, at which time no yellows had appeared in the tomato field.

In years between outbreaks, the beet leafhoppers feed on tomatoes after their migratory flights but probably find the food unsuitable, and a dispersal to other host plants occurs. During the serious 1919 and 1925 outbreaks of the pest, however, the leafhoppers were taken on tomatoes during the spring and summer. During terrific hot spells, favorable weeds of this insect often wilt and become sun-scorched—a condition which stimulates a movement to unfavorable host plants such as tomatoes, other cultivated plants, and weeds.

During 1927 other species of leafhoppers such as *Agallia californicum* Bak. and a green leafhopper, *Empoasca* sp. ?, were commonly captured on tomatoes. *E.* sp. ? was able to complete its life cycle on tomatoes.

Planting Tomatoes after Flights Cease.—Tomato-planting experiments should be conducted after the migratory flights cease in the Sacramento Valley and fog belt. The time of transplanting tomatoes may be benefited by following the late-planting schedule of sugar

beets. When beets are planted after the migratory flights end, better tonnages are obtained in years between severe outbreaks of the beet leafhopper.

When the leafhoppers are at their maximum in numbers, however, planting after the flights cease may not be entirely successful in the Sacramento Valley, since the second brood, which acquires the winged stage in July, may invade the tomato fields. During 1925, however, beets planted in the interval between the two broods made a marketable crop in the Sacramento Valley. According to J. T. Rosa, early-planted tomatoes were destroyed by yellows in 1925 on the University Farm, while late plantings made a crop.

Yaw (1924) states that "it is a common practice where plants show the disease in June to pull them out and replace with new plants in the same hole. Such plants almost never show the disease." His observations and studies as to the prevalence of tomato diseases were made mostly in the San Francisco Bay region, together with frequent trips to the lower Sacramento and San Joaquin valleys and occasional trips to the upper San Joaquin and southern California during the seasons of 1922 and 1923.

The migratory flights into the fog belt rarely occur in June except when a partial second brood develops on the foothills during years with late spring rains. Experiments should also be conducted in the fog belt to determine the time of transplanting tomatoes in relation to the end of the migratory flights of the beet leafhopper. According to Yaw, yellows usually appears in June in those plants which were set in the field in May or earlier. These observations indicate a long incubation period of the disease but do not give a clue as to when tomatoes must be transplanted to escape the beet leafhopper and curly top or yellows that it transmits.

SHADING

Where beets are grown in the shade of trees, a low percentage of curly top usually develops when the beet leafhoppers are not abundant. The leafhopper is a sunshine-loving insect and usually will not enter the shade if its food and breeding plants are favorable. During terrific hot spells favorable weeds often wilt and become sun-scorched, causing a dissemination to other food plants. In the summer of 1925 a dispersal of the adults from badly diseased beets to other host plants occurred in the Sacramento Valley as reported in a previous paper (Severin, 1926). When the food supply is suddenly cut off from the bugs by plowing under diseased beets, the adults will spread to other

food plants. These are some of the factors which stimulate the insects to enter the shade.

Humphrey (1914) noticed "that where a slight degree of shade is afforded by orchard or other trees there are relatively fewer diseased plants than where tomato plants of the same variety are grown in similar soil, but in open situations exposed to the maximum of direct sunlight."

A similar and very striking illustration was observed on the Spreckels ranch near King City in the Salinas Valley during 1926. Tomatoes grown along a fence in the shade of eucalyptus trees were, with few exceptions, healthy, while every plant exposed to sunshine was diseased. Shaded plants which appeared healthy, however, were not tested for curly top.

CLIMATIC FACTORS

Humphrey (1914) discussed the following factors in relation to tomato blight: high soil and atmospheric temperatures, wind movement, rate of evaporation and light intensity.

Shapovalov (1925, 1925a) found that a very striking correlation exists between the rate of evaporation and the prevalence and severity of the disease. He observed that climatic factors which tend to increase the evaporating power of the air, such as sunshine, temperature, humidity, and wind movement, are conducive to the development of the yellows. However, the exact manner in which high rates of evaporation facilitate the progress of the disease at that time was not clear. It is evident now, in the light of recently established etiological relationship of *Eutettix tenellus* to tomato yellows, that weather conditions are secondary factors favoring the development of symptoms and possibly the severity of the disease.

SUMMARY

The data presented in this paper prove that the beet leafhopper, *Eutettix tenellus* (Baker), transmits tomato yellows or curly top to tomatoes. Tomatoes inoculated with the curly-top virus from beets by means of infective leafhoppers developed typical symptoms of yellows under field conditions. Non-infective hoppers after feeding on the infected tomatoes were transferred to healthy sugar beets and typical symptoms of curly top were produced. The disease was also transmitted from tomatoes naturally infected with yellows to sugar beets.

Tomatoes grown in the greenhouse were susceptible to curly top, but typical symptoms of yellows failed to develop when the plants were enclosed in cages. When cages were not used, symptoms of a mild form of yellows appeared in tomatoes inoculated with the disease. The reliable symptom of sugar-beet curly top, the clearing or transparency of the veinlets, appeared in tomatoes infected with the disease in the greenhouse, but this symptom does not occur in plants naturally infected or inoculated with blight in the field.

The incubation period of the disease in the field varied from 16 to 26 days during the spring.

Curly top was also transmitted from tomatoes showing symptoms only of mosaic in a field in which both diseases were present; this transmission to beets demonstrates that the tomatoes were also naturally infected with the causal agent of curly top.

The longevity of the beet leafhoppers on tomatoes varied according to the age of the plant. The adult life of the males was shorter than that of the females on tomatoes.

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Fig. 1. White excrescences on the veins of leaflets from tomatoes infected with curly top in the greenhouse, resembling somewhat the wart-like protuberances on the leaves of diseased sugar beets.

Fig. 2. Leaflets from tomatoes infected with curly top in the greenhouse, showing different stages of yellowing between the veins, the region along the mid-rib and veins remaining green.

Fig. 1.

Fig. 2.

PLATE 2

Tomato (*Lycopersicon esculentum*)

Fig. 1. Leaf showing transparent venation on the terminal leaflets and mottling and puckering of mosaic on the lower leaflets from a plant infected with curly top and mosaic.

Fig. 2. Leaf showing mottling and puckering of mosaic from a plant also infected with curly top but not showing symptoms of tomato yellows.
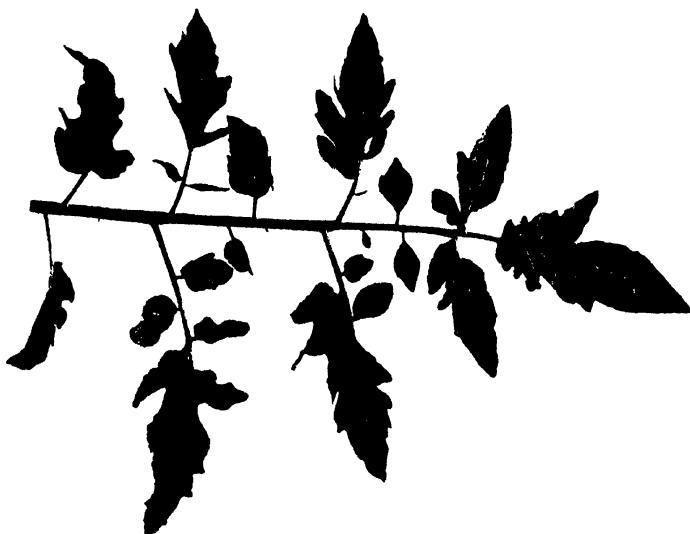
Fig. 1

Fig. 2

LETTUCE SEED AND ITS GERMINATION

H. A. BORTHWICK* AND W. W. ROBBINS†

INTRODUCTION

The germination of lettuce seed‡ (*Lactuca sativa*) is inhibited at certain temperatures, above the optimum. These temperatures may not prevent growth, however, of seeds which have already started to germinate. This inhibition seems to be largely a varietal characteristic, for the temperature at which one variety will germinate satisfactorily may completely inhibit the germination of another variety. Furthermore, it usually requires a higher temperature to inhibit the germination of old seed than it does that of freshly harvested seed of the same variety. By freshly harvested seed is meant that which is not more than five weeks old. In the case of most varieties, almost complete failure of the seed to germinate occurs at 30° C, regardless of age, although there are a few varieties that germinate fairly well at this temperature.

In the Imperial Valley of California, and in other sections with similar climate, where lettuce seed is planted in late summer and early fall, unsatisfactory stands are often obtained because of low germination. It is believed that this low germination results from high soil temperatures which prevail at germination time.

These studies have to do principally with the relation of temperatures to lettuce seed germination, but the effect of certain other environmental factors is also considered. They attempt to throw light upon the causes of inhibition of germination at high temperatures; and, they suggest practical methods of overcoming the difficulties of germinating lettuce seed at temperatures somewhat above the optimum.

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‡ By "seed" reference is made to the fruit (akene) of lettuce.

STRUCTURE OF LETTUCE SEED AS RELATED TO GERMINATION

Experiments with lettuce seed, reported later in this paper, showed that it will germinate at higher temperatures with the coats removed than with the coats on. It was found that if seeds are soaked for a half hour the coats can be separated into three distinct coverings. Removal of the outer two has no influence on the germination of seeds at high temperature but when the innermost is removed germination proceeds normally as shown in figure 23. This result suggested the desirability of making a study of the development and structure of these parts of the seed.

Jones[2] describes the general morphology of the lettuce akene, but gives no details as to the development of the pericarp and other coats surrounding the embryo.

Figures 1 and 2 show sections of the ovary and ovule cut through the embryo sac just prior to anthesis. The embryo sac is highly vacuolate and has a single nucleus. The nucellus has almost entirely disappeared, being represented now by a few scattered cells. The single integument consists of from twelve to eighteen tiers of parenchymatous cells, the inner nutritive layer being particularly prominent because of the large size of its cells. There is evidence, at this stage, of disorganization of certain inner cells of the integument (except the epidermis). The pericarp varies in thickness in different parts of the akene. The cells of the pericarp resemble those of the integument, except that on the whole they are smaller; the outer epidermis of the pericarp is distinct but there is no well-defined inner epidermis. Inner pericarp cells have begun disorganization which process progresses with greater speed at certain places than at others, giving rise to large lysigenous spaces adjacent to the integument.

Examination of figures 3 and 4, sections of akenes older than those described above, shows that there has been an enlargement of the akene, which involves in part an increase in the thickness of the integument. There has been further disorganization of inner pericarp and inner integument cells. Figures 5 to 14 show still further disintegration of pericarp and integumentary tissue, enlargement of the embryo sac and embryo, and organization of two endosperm cell layers contiguous to the inner epidermis of the integument.

The inner epidermal cells of the integument finally disorganize (figs. 14, 15, 16) ; fragments of walls are in evidence, however, as late as ten days after anthesis. Integumentary cells along the sides of the

embryo sac disappear sooner than those at the ends. The inner wall of these epidermal cells becomes a prominent structure in the mature seed. This wall closely invests the endosperm, and is with difficulty distinguished from it. That it is of integumentary origin is well shown in figures 15 and 16. This membrane is a continuous one, which in untreated sections, appears translucent in color. It has semi-permeable properties. It stains yellowish red with Sudan III, dissolves in strong hot potassium hydroxide, but is insoluble even in concentrated sulphuric acid. These reactions show it to be fatty in nature. When the pericarp and seed coats of a mature akene are removed, and the embryo, surrounded by the unbroken integumentary membrane and the endosperm, is immersed in concentrated sulphuric acid, the former is thrown into folds and separates from the endosperm. The sulphuric acid apparently dissolves the walls of the endosperm cells and the dissolved substances are obviously highly osmotic for if the seeds are now immersed in distilled water, absorption is rapid, the membrane becomes greatly distended, and finally bursts, the points of rupture usually occurring along the side of the seed. This behavior indicates that the membrane is continuous. When the embryo surrounded by the endosperm and the integumentary membrane is immersed for a short time in hot potassium hydroxide, and then transferred to distilled water, swelling occurs as with the sulphuric acid treatment, but the membrane ruptures much quicker and always near the root tip. The point of rupture corresponds to the position of the micropyle.

The structure of the tissues which surround the embryo of the mature akene may be summarized as follows:

(1) The *pericarp* has rather equally spaced ribs (figs. 1, 3, 8, and 11) which are made up of thick-walled sclerenchymatous pitted cells (figs. 17, 18, 19) which give a strong lignin reaction. In longitudinal sections, these cells appear much like fibers. The pericarp cells between the ribs are somewhat larger, and thinner walled than those of the ribs, but also lignified to some extent. Pericarp cells present in the mature akene represent only a part of those found in the young akene; inner pericarp tissue disorganizes in the course of akene development.

(2) The *integument* is composed of (*a*) a persistent outer epidermis with thick walls (fig. 19), (*b*) remnants of disorganized cells, and (*c*) a conspicuous suberized semi-permeable membrane (fig. 20), which belongs to the wall of the inner epidermis of the integument adjacent to the endosperm. During the development of the seed, most cells of the integument are disorganized.

(3) The *endosperm,* in most parts of the seed consists of a distinct layer two cells thick (fig. 19) ; at the root end the layer is often three or more cells thick (fig. 20). These cells are thick-walled, with here and there wall projections (fig. 21) into the cell lumen. The projections vary in length, sometimes mere pegs, whereas in other instances they extend from wall to wall, being of the nature of trabeculae. Endosperm cell walls are not lignified, and the lumina are filled with fatty and proteinaceous substances.

During the development of the akene, although there is progressive disorganization and dissolution of the inner part of the pericarp, as well as of the inner part of the integument, the pericarp and the integument are brought very close together by pressure from within, resulting from the enlargement of the embryo.

These morphological studies show that the three parts into which the coats of a mature akene may be separated are (*a*) the remains of the pericarp; (*b*) the outer epidermis of the integument with remnants of disorganized cells of the integument; and (*c*) the membrane from the inner epidermis of the integument together with the endosperm.

PHYSIOLOGICAL STUDIES

Seed Used.—The seed used in these experiments was in most cases the variety known as New York, for it is this which is grown almost exclusively for market in the principal lettuce areas of California. Other varieties were also used. Most of the seed was obtained from C. C. Morse and Company, San Francisco, and was produced by them either near Hollister or Sacramento, California. Seed produced on the University Farm was used in a few experiments, in which freshly harvested seed was needed. The commercially produced seed was used wherever possible, however, because it was graded better than hand-cleaned seed and therefore gave a higher percentage of germination.

Methods.—Experiments were carried out both in the laboratory and in the field. Petri dishes were used as germinating dishes in certain of the laboratory experiments. In each dish was placed a double layer of Canton flannel which was saturated with water and then allowed to drain by inverting the dish for a short time. This made the water content of the different germinators reasonably uniform. The seeds were sprinkled loosely on this moist cloth and the dishes were covered and held at the proper temperatures.

Other experiments were made in the laboratory using flats of garden soil. The flats of soil were placed in a large room, the tem-

perature of which was kept constant within one degree centigrade. An electric fan was installed to keep the air circulating. The seeds were planted about one-fourth inch deep in rows of 100 seeds each. The soil was kept thoroughly moist. Most of these experiments were replicated ten times with controls planted in the same flat with the treated seeds.

Field experiments were conducted both at Davis and at Meloland, Imperial County. The seeds were planted on ridges, imitating commercial practices as closely as possibe.

Influence of Temperature upon Percentage of Germination.— Lettuce seed germinates very well over a considerable range of temperature, but the percentage germination falls off very abruptly at temperatures above 25° C as is shown in table 1.

TABLE 1

PER CENT GERMINATION OF LETTUCE SEED (NEW YORK) AT VARIOUS TEMPERATURES
(Seed 18 months old)

Germination temperature in degrees Centigrade ..	1°.	4°	17°	20°	22°	25°	27°	28°	29'	30°
Per cent germination	99	99	98	98	99	98	76	20	2	0

Seed of the same variety which was only four months old showed a similar behavior but the reduction in germination occurred at a slightly lower temperature (table 2).

TABLE 2

PER CENT GERMINATION OF LETTUCE SEED (NEW YORK) AT VARIOUS TEMPERATURES
(Seed 4 months old)

Germination temperature in degrees Centigrade	1°	17°	20°	22°	25°	26°	29°	30°
Per cent germination	99	98	98	93	83	4	0	0

It is probable that the differences shown in tables 1 and 2 are correlated with the age of the seed. In this connection it should be stated that it is the practice of lettuce growers in sections where lettuce is sown under high temperature conditions to plant seed that is twelve months, or more, old, rather than that produced the current season. It should be mentioned here also, that in cases like those shown in tables 1 and 2, where seeds germinate satisfactorily at low temperatures, but not at high temperatures, the ungerminated seeds are in no way injured by the high temperature. They appear to be

as fully imbibed with water as those which germinate. They have been known to remain in this condition for weeks without showing any decay and still germinate well as soon as the temperature is reduced. Data pertaining to this point will be given in another section of this paper.

It will be seen from tables 3 and 4 that varieties differ greatly in the degree of temperature which they can tolerate without any reduction in germination.

TABLE 3

GERMINATION OF DIFFERENT VARIETIES OF LETTUCE SEED ABOUT FOUR MONTHS AFTER HARVEST

(Seed from C. C. Morse and Co.)

Variety	Per cent germination		
	12° C	25° C	29° C
	per cent	*per cent*	*per cent*
All Year Round	99	95	36
All Year Round	97	96	84
Big Boston	97	54	0
Black Seeded Simpson	100	82	29
Deacon	98	67	35
Denver Market	97	58	26
Drumhead	99	96	89
Early Curled Simpson	96	21	0
Grand Rapids	98	95	85
Hanson	92	80	3
Hardy Green Winter	95	89	23
Hicks Hardy White Winter Cos	99	67	8
Hubbard's Market	98	59	0
Iceberg	99	97	92
Mammoth Black Seeded Butter	98	97	48
Mammoth Black Seeded Butter	92	88	31
May King	94	87	7
New York	99	91	1
Prize Head	99	81	1
Salamander	98	96	88
Tom Thumb	97	97	89
Paris White Cos	98	97	63

It will be noticed in table 3 that all varieties gave higher than 90 per cent germination at 12° C, showing that the seed was of high germinating capacity. At 25° C, however, the germination was reduced in twelve varieties to below 90 per cent, while at 29° C only one variety gave over 90 per cent germination. The amount of reduction caused by raising the temperature from 12° C to 29° C varied greatly with different varieties. Six varieties showed less than 15 per cent reduction, as seen from the table, while eight showed a reduction of over 85 per cent germination. New York falls in the group which is the most sensitive to high temperature.

TABLE 4

GERMINATION OF DIFFERENT VARIETIES OF LETTUCE SEED IMMEDIATELY AFTER
HARVEST

(Seed grown on University Farm, Davis)

Variety	Per cent germination		
	8° C	12° C	22-25° C
	per cent	*per cent*	*per cent*
Big Boston	44	37	1
Black Seeded Simpson	66	62	22
Black Seeded Simpson	42	41	20
California Cream Butter	14	18	3
Chicken Lettuce	40	34	2
Denver Market	67	71	10
Early Curled Simpson	32	24	1
Grand Rapids	55	57	15
Hanson	28	35	3
Hubbard's Market	15	13	1
Iceberg	52	54	58
Iceberg	61	60	58
Malta	12	14	4
May King	94	95	0
New York	67	69	8
Paris White Cos.	91	94	6
Prize Head	49	41	9
Unrivaled	39	46	2
Wayahead	45	51	4

Influence of Dry Storage at Different Temperatures upon Subsequent Germination.—In order to have seed available as quickly as possible after maturity, small quantities of seed from many varieties of lettuce were harvested and cleaned by hand. The percentage at germination was determined immediately for each variety. These results are shown in table 4. In this table it will be seen that there is no significant difference in germination of any of the varieties at 8° and 12° C, from which it would appear that 12° is sufficiently low for satisfactory germination. On the other hand almost every variety that showed any appreciable germination at these low temperatures showed a marked reduction at 22°–25° C, except Iceberg. This shows again the inhibiting influence of high temperature upon the germination of lettuce seed. The seed from each variety was then divided into three parts and placed in open wide-mouthed bottles for storage. Two of these lots were kept at 4° and 12° C, respectively, in constant temperature chambers while the third was kept in the laboratory at about 20° C. At frequent intervals during a storage period of 37 days, seed from each of these lots was germinated at 12° and

TABLE 5

PER CENT GERMINATION OF LETTUCE SEED AT DIFFERENT TEMPERATURES AFTER
VARIOUS PERIODS OF STORAGE (DRY)

(Seed grown at University Farm, Davis)

Variety	Germinated at 12° C												
	Stored 0 days	Stored 7 days			Stored 13 days			Stored 20 days			Stored 37 days		
		4° C	12° C	Lab.	4° C	12° C	Lab.	4° C	12° C	Lab.	4° C	12° C	Lab.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Big Boston	37	7	26	47	43	28	35	45	24	45	41	31	40
Black Seeded Simpson	62	69	70	68	67	60	66	45	66	67	83	66	66
Black Seeded Simpson	41	36	39	52	51	38	45	45	43	47	42	42	54
California Cream Butter	18	28	8	24	27	25	12	23	20	25	25	29	8
Chicken Lettuce	34	37	47	41	37	50	38	31	44	37	36	45	32
Denver Market	71	74	71	68	70	63	74	67	75	75	70	73	60
Early Curled Simpson	24	16	19	18	23	19	33	28	23	27	22	20	37
Grand Rapids	57	58	63	61	58	61	59	49	59	68	45	56	58
Hanson	35	48	42	32	38	27	33	29	32	37	36	40	41
Hubbard's Market	13	23	30	25	19	26	16	18	25	12	18	24	16
Iceberg	54	50	48	73	58	50	57	51	51	58	49	45	66
Iceberg	60	55	63	71	62	58	82	62	52	72	61	50	65
Malta	14	18	37	7	22	14	5	14	17	7	13	21	16
May King	95	93	95	95	96	96	92	94	94	93	95	94	93
New York	69	45	56	67	58	53	63	62	55	64	57	50	70
Paris White Cos	94	95	95	92	92	93	89	93	91	92	95	93	93
Prize Head	41	45	40	47	45	44	53	39	35	58	44	42	47
Unrivaled	46	64	52	41	66	50	53	63	48	65	61	58	66
Wayahead	51	51	48	55	52	48	47	51	44	57	52	45	52

Variety	Germinated at 25° C												
	Stored 0 days	Stored 7 days			Stored 13 days			Stored 20 days			Stored 37 days		
		4° C	12° C	Lab.	4° C	12° C	Lab.	4° C	12° C	Lab.	4° C	12° C	Lab.
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Big Boston	3	1	4	6	4	4	5	6	7	20	10	10	7
Black Seeded Simpson	37	37	36	52	26	51	48	39	61	65	54	52	68
Black Seeded Simpson	22	24	23	36	31	38	21	28	28	41	20	25	36
California Cream Butter	5	13	3	13	10	10	7	8	14	15	5	11	5
Chicken Lettuce	3	2	5	5	7	15	4	5	14	17	3	12	25
Denver Market	10	16	15	23	21	22	24	17	30	45	26	30	35
Early Curled Simpson	3	4	4	1	8	4	5	6	6	8	4	11	10
Grand Rapids	16	28	27	42	26	33	26	22	52	56	31	53	52
Hanson	10	7	11	10	10	8	8	10	17	32	11	18	24
Hubbard's Market	1	3	6	5	4	6	7	7	5	8	4	5	12
Iceberg	49	50	44	55	49	48	66	45	47	48	50	52	55
Iceberg	66	66	58	72	52	49	81	57	53	62	51	53	68
Malta	9	11	22	5	12	21	5	10	18	6	10	17	9
May King	2	1	2	3	7	4	7	1	5	19	5	4	8
New York	10	18	15	25	30	21	25	27	32	58	23	36	59
Paris White Cos	6	19	18	42	54	43	47	63	69	84	64	79	75
Prize Head	13	13	14	12	12	24	10	15	20	44	17	31	37
Unrivaled	3	8	8	7	14	15	7	7	24	42	2	19	45
Wayahead	5	10	8	17	12	16	15	13	27	39	11	27	28

25° C.   In table 5 are given these germination results obtained on five successive dates.   It will be noted that at a germination temperature of 12° C the results obtained at the end of 37 days are not significantly better than those immediately after harvest.   At 25° C, however, where the germination immediately after harvest was considerably below that at 12° C, a gradual increase was found in many of the varieties during the period of storage at the temperatures employed.

In no case where seed is germinated at 12° C is there any evidence of improved germination resulting from storage at 4° or 12° C as compared with the storage at laboratory temperature, with the possible exception of Unrivaled and one lot of Black Seeded Simpson stored at 4° C.   In other words, the improvement in the germination of the seed after a period of storage (dry) is due to the natural aging of the seed, rather than to the effect of temperature.   The results would seem to indicate that dry storage of lettuce seed at low temperatures is no more effective in improving germination than dry storage at ordinary temperatures.

Effect upon Vitality, of Storage (Moist) at Temperatures Which Inhibit Germination.—Moist seeds which are exposed to temperatures of 30° C or slightly above do not germinate even though other conditions are suitable.   The seeds are not injured, however, for when transferred to low temperatures, good germination may be secured. Lots of seed were kept under germinating conditions at 30° C from 1 to 13 days after which they were placed at 16°–18° C.   The germination each day after they were placed at the lower temperature is recorded in table 6.

TABLE 6

PER CENT GERMINATION OF LETTUCE SEED AT 16–18° C AFTER VARIOUS PERIODS
UNDER GERMINATION CONDITIONS AT 30° C

Days stored at 30° C	Per cent germination			
	1 day at 16–18° C	2 days at 16–18° C	3 days at 16–18° C	4 days at 16–18° C
0	....	99	....	....
1	97	....	....	..
2	76	92	99	....
3	62	95	99	....
4	5	76	95	....
6	0	30	88	100
7	0	26	67	98
10	0	68	91	99
13	0	3	64	98

It is to be noted that, although satisfactory germination takes place after the temperature is lowered, recovery is slower the longer the exposure to 30° C. Similar results are reported by Davis[1] who states that lettuce seeds which remained dormant on moist cotton for eight months at 27° to 30° C germinated almost completely in ten days when exposed to fluctuating laboratory temperatures. It appears from the results obtained by the writers that the failure of seeds to germinate at 30° C is not the result of temperatures too high for growth of the embryos, for naked embryos make normal growth at 30° C or at somewhat higher temperatures. At 40° C however, root growth is inhibited, although the hypocotyl and cotyledons make considerable growth even at this latter temperature.

Why Does Lettuce Seed Fail to Germinate at Temperatures which are Favorable to Seedling Growth?—Since 30° C is evidently not too high for growth of lettuce seedlings, experiments were made to determine the reason for the failure of seeds to germinate at this temperature. All the coverings were removed from a number of seeds after a few hours soaking and the naked embryos were placed at 30° C on moist germinators. In 24 hours, in almost every case, growth was apparent as shown in figure 23. When, however, the pericarp and the outer part of the seed coat only were removed leaving the embryo still enclosed by the endosperm and integumentary membrane, no germination occurred, indicating that one or both of these structures prevents germination at high temperatures. Seeds which have been at 30° C for as long as five days without germination will germinate in a few hours at this same temperature after the endosperm and integumentary membrane are removed.

It appears that the first stages in lettuce seed germination are initiated, or at least proceed most satisfactorily, only at low temperatures, providing there is adequate moisture and oxygen. If seed is exposed to the foregoing conditions for varying periods, even though there are no visible signs of germination except swelling of the seed, and then transferred to higher temperatures, germination proceeds normally. The situation just described may also be expressed in another way: at temperatures of approximately 28° C and above, the initial stages of germination are inhibited. As was shown above, the cause of this inhibition may be traced to the tissue which so closely invests the embryo. Absorption of water is not hindered. Davis[1] has shown that in from four to six hours, at temperatures from 20° C to 35° C, the seed absorbs sufficient water for germination. He gives evidence however that the integumentary membrane prohibits the free diffusion of oxygen inward. The oxygen requirements increase rapidly

with an increase in temperature, such that an adequate supply fails to diffuse through the membrane at the higher temperatures. Davis[1] states that "when lettuce seed is maintained at a temperature too high to permit of germination, the seed coats gradually become less permeable to gasses as is indicated by a marked falling off in the respiratory intensity." Evidence is also given that this same membrane prevents the free diffusion of carbon dioxide outward. Davis[1] states that carbon dioxide is restricted in its diffusion to a less extent than oxygen. There is also the possibility, as suggested by Sifton,[3] in the case of spinach seed, that there are deleterious products of metabolism in the endosperm or embryo, arising, and possibly accumulating, only at the higher temperatures which inhibit the initial germination stages. The fact remains that the naked lettuce embryo germinates at high temperatures, whereas with the endosperm and integumentary membrane intact, it does not.

Further evidence indicating that the failure of seeds to germinate is caused by insufficient oxygen reaching the embryo, was obtained by germinating seeds in increased pressures of oxygen. The chambers used in the experiments consisted of cylindrical battery jars of 4½ liters capacity inverted in a shallow glass dish about 2 inches in depth. The chamber was large enough so that the respiration of 100 seeds would not greatly alter the composition of the atmosphere within.

The percentages of oxygen indicated in table 7 are the approximate amounts which were placed in the germinating chambers at the beginning of the experiment.

TABLE 7

PER CENT GERMINATION OF LETTUCE SEED AT 30° C IN ATMOSPHERES OF VARIOUS
OXYGEN CONCENTRATIONS

Oxygen content of atmosphere (per cent in germinator)	20	29	37	46	50	60	72	80	90
Per cent germination	2	1	3	9	10	1	7	38	33

Figure 22 shows young seedlings (*a, b, c*) which grew at ordinary oxygen pressure and an optimum temperature, and also seedlings (*d, e, f*) which grew at a high oxygen pressure and 30° C. Under the latter conditions, cotyledonary growth is not retarded whereas radicle and hypocotyl growth is inhibited. The growth habit and structure of seedlings at 30° C and ordinary oxygen pressure are normal.

Germination at High Temperatures after Exposure of Moist Seed to Low Temperatures for Varying Lengths of Time.—It has already been mentioned that lettuce germinates well at low temperatures and

that growth of seedlings is not retarded by a temperature of 30° C. Experiments were therefore conducted to determine how soon seeds could be transferred from germinating conditions at various low temperatures to 30° C without having the higher temperature cause a reduction in germination.

In table 8 are given some of the results of laboratory experiments using Petri dishes.

TABLE 8

FINAL PER CENT GERMINATION OF NEW YORK LETTUCE SEED AT 30° C AFTER
EXPOSURE (MOIST) TO DIFFERENT TEMPERATURES FOR VARYING PERIODS
(Seed harvested the preceding season and germinated in Petri dishes)

Temp., degrees C. at which exposed	Hours exposed						
	0	7	12	16	24	48	72
1°..	0	....	....	....	13	20	65
4°..	0	...	....	....	...	93	97
16-18° C	0	30	72	71	87	....	....
16-18° C (freshly harvested seed)	0	1	15	48	74	....	...

At the time the above lots of seed were transferred to the germinator at 30° C there was no visible evidence of germination.

The following experiments were carried out using flats of soil in which to germinate the seeds. The germination temperature was 30° C. The treatment consisted of storage at 4° C, moist, for six days. After storage the seeds were dried sufficiently so that they would not stick together, then counted and planted. Ten lots of 100 treated seeds each and a similar number of untreated seeds, as checks, were used in each case (fig. 24). The results are shown in table 9.

TABLE 9

PER CENT GERMINATION IN SOIL FLATS AT 30° C AFTER SIX DAYS STORAGE
(MOIST) AT 4° C

Source of seed	per cent germination	
	Treated	Untreated
New York (1927) University Farm, Davis...	71	0.2
Prize Head (1926) C. C. Morse and Company...........................	67	0.7
New York (1927) C. C. Morse and Company..........................	67	0.6

Other experiments were carried out in a similar manner except that the seeds were allowed to dry out for longer periods of time after treatment. These also, with the controls, were replicated ten times (table 10).

TABLE 10

PER CENT GERMINATION IN SOIL FLATS AT 30° C AFTER SIX DAYS STORAGE (MOIST)
AT 4° C, FOLLOWED BY VARYING PERIODS OF DRYING
(Variety, New York, 1927, C. C. Morse and Co.)

	Per cent germination			
	Dry 2 hours	Dry 2 days	Dry 6 days	Dry 14 days
Treated..............	67	57	39	32
Controls......	0.6	0 7	0 4	0 5

In field tests at Davis twelve lots of 100 seeds each of 1-year-old Prizehead, untreated, and twelve lots which had been kept moist at 4° C for six days were planted in moist soil in ridges so that they could be irrigated. The results are as follows: The average per cent of germination of the twelve lots in warm soil after storage, moist, for six days at 4° C was 65 per cent; whereas, the average of a similar number of lots of untreated seed was 30 per cent. Soil temperatures at the level of the seeds reached 30° C between 9 and 10 o'clock and remained above that point until after 4 P.M. The maximum recorded for this period was 39° C. Seedlings from seeds stored moist at low temperature were appearing in abundance a day before those from the untreated seeds.

In the field tests conducted in the Imperial Valley (1927) near Meloland, seed of the New York variety, 1927 harvest, from C. C. Morse and Company was used. The treatment in each case was as follows: the seeds were placed between the folds of moist cheese cloth, and kept on ice for five days. The temperature range was from about 3° to 5° C. Before planting, the seeds were dried until they would not stick together. The plantings were made on ridges, following the methods employed by commercial growers. The ridges were moist when the seed was planted. The results are shown in table 11.

TABLE 11

PER CENT GERMINATION IN THE FIELD OF SEED STORED MOIST ON ICE FOR FIVE
DAYS
(Planted October 10, 1927)

Treatment	Per cent germination			
	After 2 days	3 days	4 days	5 days
Control (untreated) (16 lots).............	1	2	7	14
Treated, planted immediately (11 lots)............	33	59 .	....	62
Treated, dry 2 hours (9 lots)...............................	16	56	... .	76
Treated, dry 24 hours (5 lots)...............................	24	43	51	....

Reference has been made repeatedly to the storage of seed, *moist*, at low temperatures. These conditions provide for good aeration of the seed. The question arises in this connection whether or not the seed cannot just as well be immersed in water at low temperatures. Tests show that this treatment is followed by very poor germination. This is particularly true if large quantities of seed are employed, such that poor aeration is secured.

DISCUSSION AND SUMMARY

The requirements for the germination of lettuce seed are an adequate supply of moisture, a low temperature (below 25° C), and good aeration. Coats surrounding the embryo do not limit the intake of water. Seeds absorb sufficient water for germination in from four to six hours. High percentages of germination are secured over a wide temperature range, from 1° to 25° C. At temperatures between 25° and 30° C most varieties of lettuce fall off rapidly in percentage germination; at 30° C in most varieties, germination is almost entirely inhibited.

Different varieties of lettuce seed grown under similar conditions and of the same age vary, however, in their response to high temperatures. Some varieties attain fairly high percentage germination at 29° C; others have very low germination at this temperature.

Although there is a varietal difference in the response of lettuce seed to high germination temperatures, these differences disappear when the seed is germinated at low temperatures.

Generally speaking, freshly harvested lettuce seed is inferior in its germinating power to that several months old. With many varieties, the germination at ordinary temperatures improves noticeably in the first few weeks after harvest.

The storage of lettuce seed, dry, at low temperatures for periods of from 7 to 37 days did not improve its germination, as compared with seed stored at laboratory temperatures.

Lettuce seed may be kept at 30° C, either dry or moist, without altering its ability to germinate when placed at a lower temperature. Davis[1] reports that he "kept lettuce seed upon absorbent cotton continuously for a period of eight months at a temperature from 27° to 30° C without perceptible loss of seed."

Within the limits of the experiments here reported the longer the exposure of the seed (moist) at 30° C, the longer the time required for germination when placed at a lower temperature.

The failure of lettuce seed to germinate at temperatures of approximately 30° C and above is ascribed to the inhibiting influence exerted

by a structure which closely invests the embryo. This structure includes the endosperm (two layers of cells) and a semi-permeable integumentary membrane. There is evidence that this structure retards gas exchange. The oxygen requirements at high temperatures are greater than at low. Increased oxygen pressure increases the germination percentage. There is also the possibility that there are products of metabolism arising and probably accumulating in the endosperm or embryo at high temperatures, and that these products inhibit initial germination stages.

If the early stages of germination are initiated at low temperatures, growth is uninterrupted by the transference of the seed to high temperatures.

If seed is to be planted in a soil which has a temperature during many hours of the day, of 30° C or above, a treatment as follows is recommended as a practical measure: Store *moist*, with *good aeration* at approximately 4° C for a period of four to six days. Soaking the seed in water at this temperature for any length of time is ineffective, because the seeds do not have sufficient aeration. Practically, this is accomplished by placing the seed between the folds of moist burlap and storing on ice.

Seed that has been treated as recommended above may be thoroughly dried at room temperature with no appreciable loss in viability. One lot of treated seed gave a germination of 32 per cent after fourteen days drying at room temperature, whereas the untreated check gave a germination of 0.5 per cent. These experiments suggest the probability that certain changes initiated at these low temperatures, when moisture and oxygen are adequate, are irreversible.

ACKNOWLEDGMENTS

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PLATE 1

Fig. 1. Diagrammatic cross-section of lettuce ovary two hours before anthesis.

Fig. 2. Cross-section of portion of lettuce ovary, two hours before anthesis.

Fig. 3. Diagrammatic cross-section of lettuce ovary, twenty-six hours after anthesis.

Fig. 4. Cross-section of ovary wall, and seed coats of lettuce, thirty-four hours after anthesis.

· NOTE.—In the plates following three different magnifications were employed. Figs. 1 to 21 underwent the same reduction. Figs 1, 3, 5, 8 and 11 are × 47; Figs. 2, 4, 6, 7, 10, 12, 13, 14, 15, 16, 17, 19 and 20 are × 237; Figs 9 and 18 are × 245.
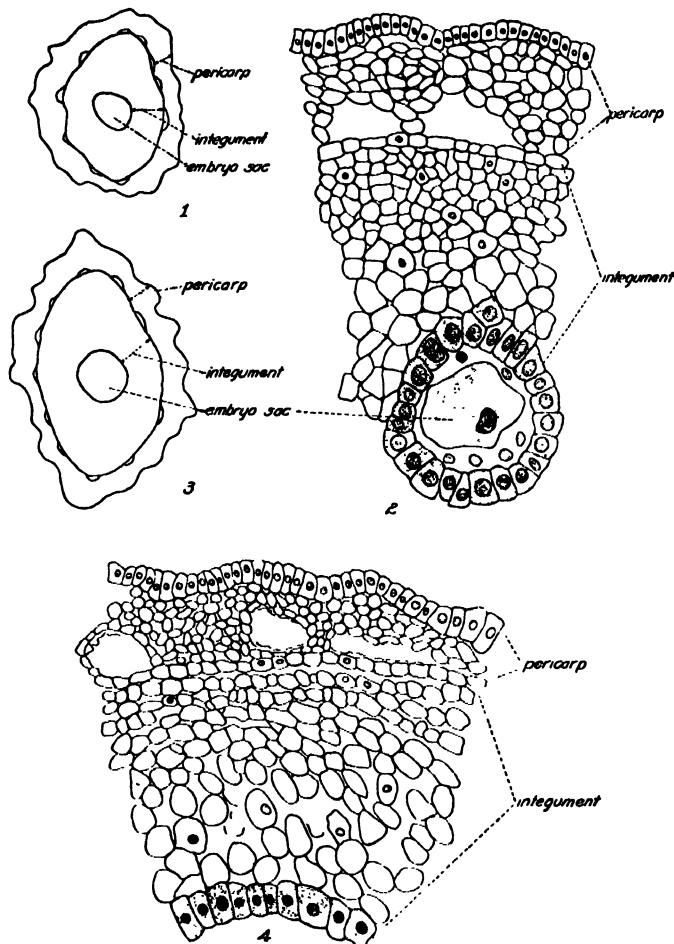
PLATE 2

Fig. 5. Cross-section of lettuce ovary, three days after anthesis.

Fig. 6. Section of embryo sac.

Fig. 7. Cross-section of a portion of lettuce ovary, three days after anthesis.
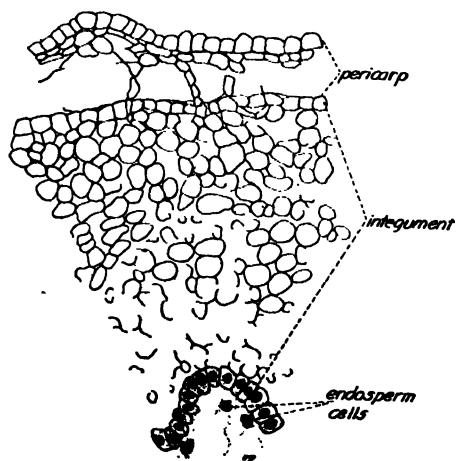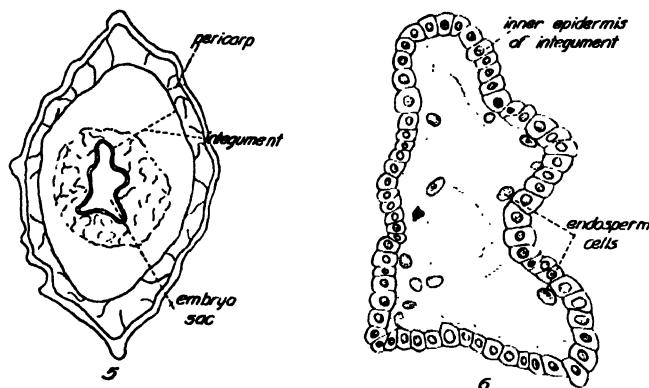
PLATE 3

Fig. 8.   Cross-section of lettuce ovary, four days after anthesis.

Fig. 9.   Same as figure 8, showing details of a portion.

Fig. 10.   Cross-section of lettuce ovary, five days after anthesis.

inner epidermis
of integument

endosperm
cells

cotyledons

9

pericarp

integument

cotyledons

8

pericarp

outer epidermis
of integument

inner epidermis
of integument

outer cell layer
of endosperm

free endosperm cells

10
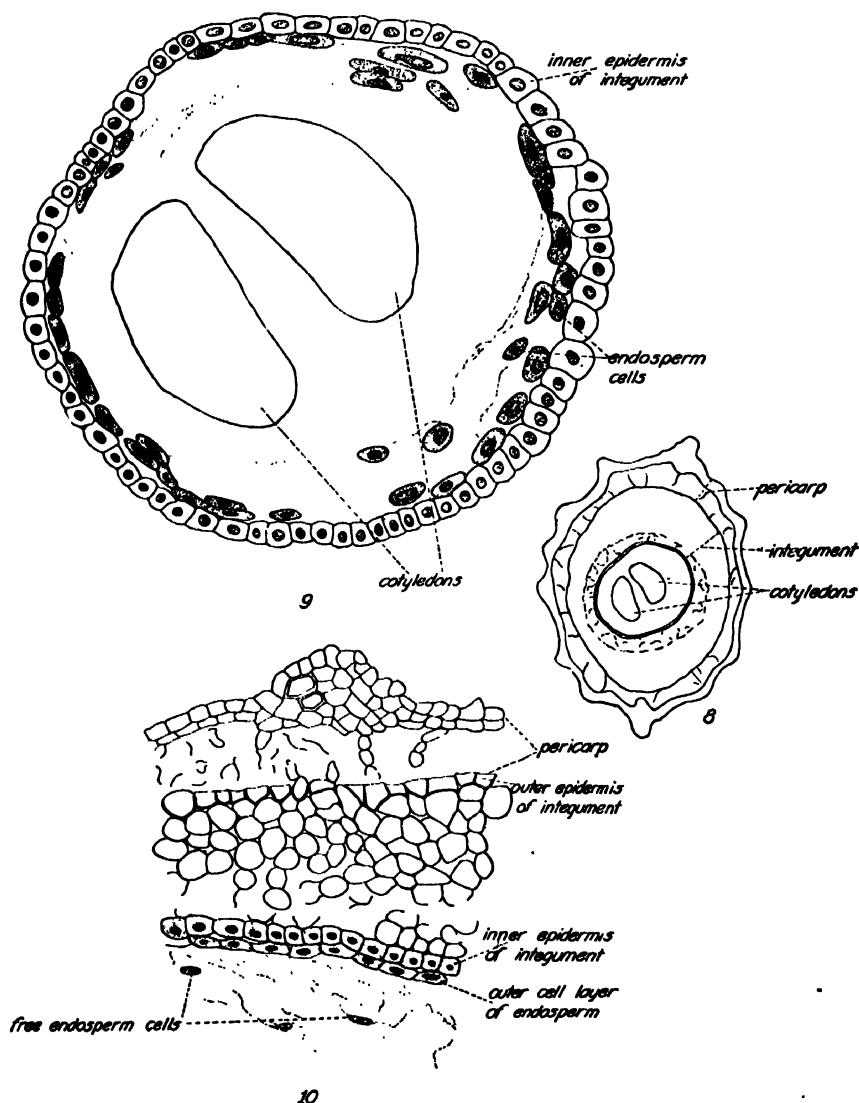
PLATE 4

Fig. 11. Cross-section of lettuce akene, seven days after anthesis.

Fig. 12. Same, showing detail of structure through stem growing point.

Fig. 13. Same, through root tip.

Fig. 14. Portion of endosperm, showing disintegrating epidermis of integument, and suberized membrane, eight days after anthesis. Section taken alongside of embryo.
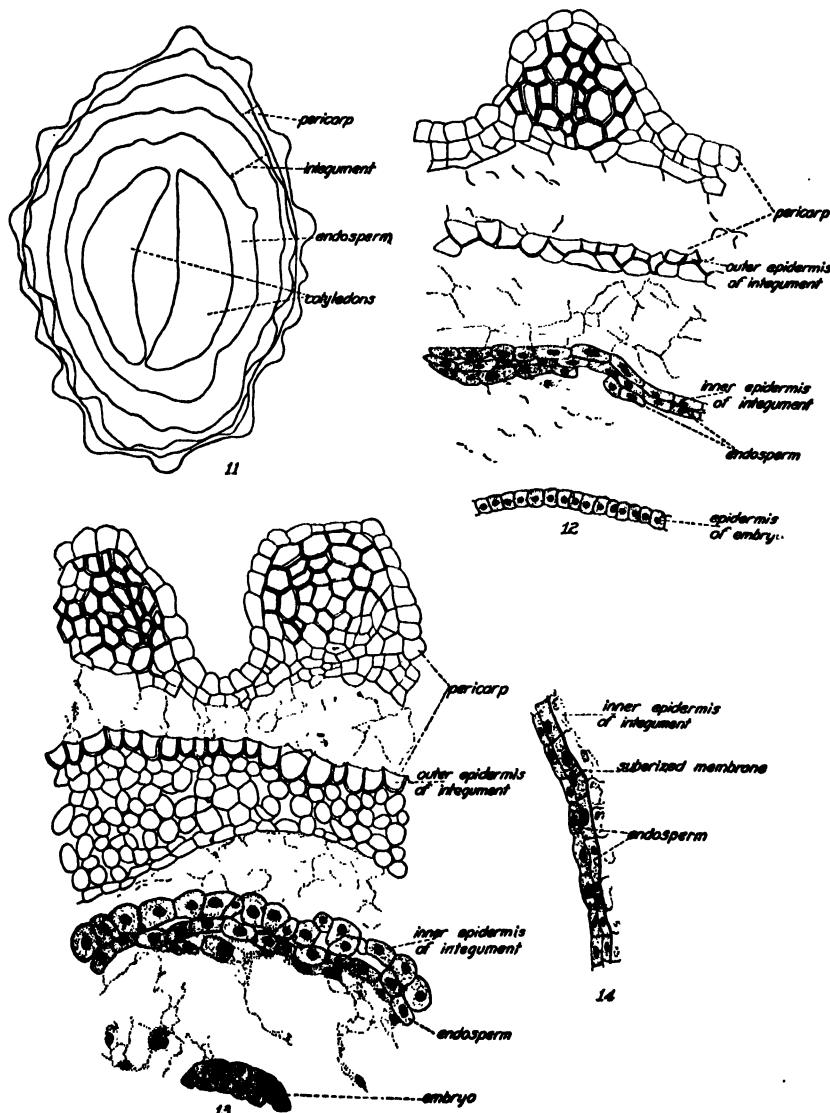
PLATE 5

Fig. 15. Portion of endosperm and inner epidermis of integument, ten days after anthesis. Section taken near root tip.

Fig. 16. Same as preceding, showing further disintegration of walls.

Fig. 17. Cross-section of a portion of akene, ten days after anthesis, inner epidermis of integument has disappeared. Section taken near middle of seed.

Fig. 18. Lengthwise section of a portion of akene, ten days after anthesis.
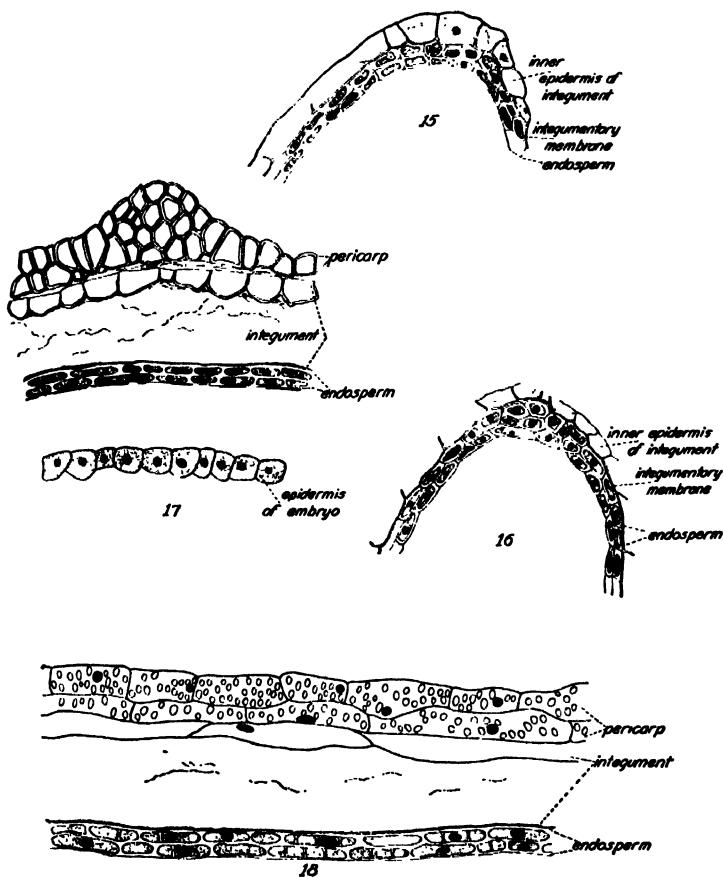
PLATE 6

Fig. 19. Cross-section through the coats of mature lettuce akene.

Fig. 20. Cross-section of endosperm of mature lettuce akene, cut through the radicle of the embryo.

Fig. 21. Surface view of endosperm cells. The dotted lines indicate the walls of the lower layer of endosperm cells.

--pericorp
---integument

19

endosperm
epidermis of
embryo

integumentary
membrane

20

endosperm

21
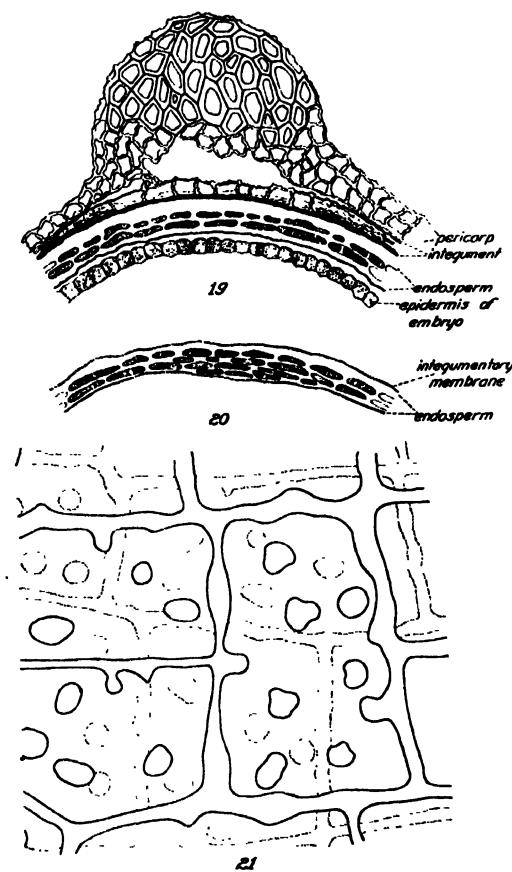
Fig. 22.   Early stages in the germination of lettuce seed; *a*, *b*, and *c*, grown at ordinary oxygen pressure at 30° C, *d*, *e*, and *f*, grown under high oxygen pressure at 30° C.   In the latter, note that radicle and hypocotyl growth is retarded, whereas cotyledonary growth is not retarded.
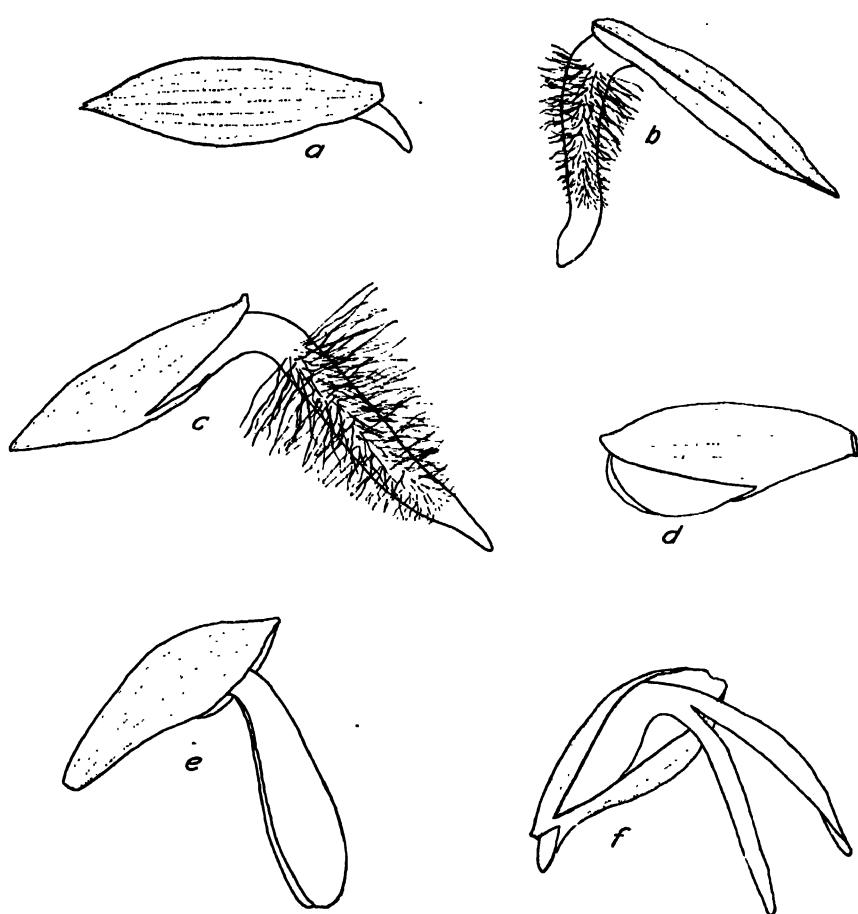
PLATE 8

Fig. 23. Akenes after 36 hours at 30° C, with ample moisture and oxygen. Upper row: endosperm intact, but pericarp and integuments removed; lower row: all coats, including endosperm, removed.

Fig. 24. Influence of the exposure of lettuce seed, moist, to low temperature, upon its subsequent germination at 29° C. Variety, Prize Head, about 1 year old. Left, seeds kept moist at 4° C for 6 days, between folds of cloth, and then planted in garden soil at 29° C; 674 seedlings from 1,000 seeds. Right, seeds kept dry at laboratory temperature and then planted in garden soil at 29° C; 7 seedlings from 1,000 seeds. Seeds planted Sept. 14; count and photograph taken Sept. 17.
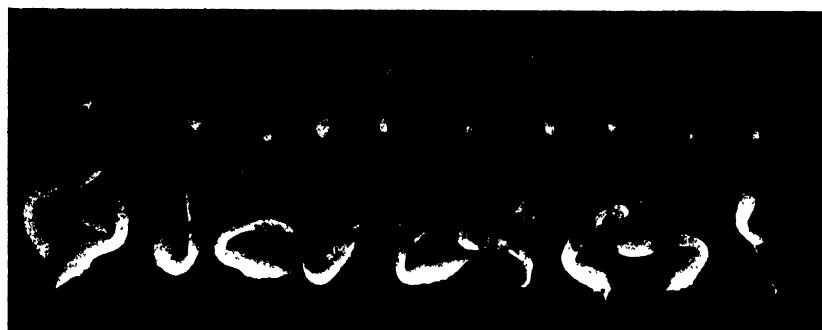
Fig. 23.
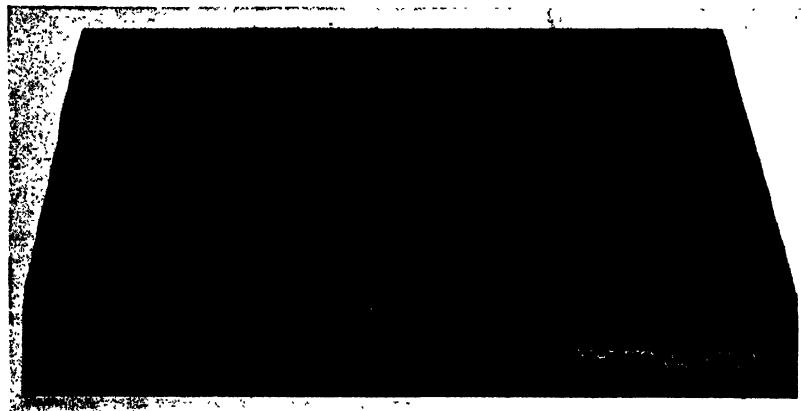
Fig. 24.

FACTORS AFFECTING THE PRICE OF WATERMELONS AT LOS ANGELES

EMIL RAUCHENSTEIN[1]

THE PROBLEM

As the quantity of any commodity put on the market increases, the value which the consumer places on each unit declines, and hence he will pay less for each unit. It is the purpose of this study to determine for a specific commodity at a specific market (watermelons on the Los Angeles market) how much prices have actually changed during the past six years with the various changes in the supply, and to measure the effect of all other factors on which data are available and which affect the price.

It is a matter of general experience that variations in the supply of one commodity may cause large proportional changes in its price, whereas similar variations in the supply of another commodity may cause only small proportional changes in its price. For example, an increase of 20 per cent in the supply of potatoes would cause a large relative decrease in their price.[8] A similar increase in the supply of apples would cause a much smaller proportional decrease in their price.[5] It is possible also that the demand for a commodity may change over a period of time. The price of potatoes seems to change more now for a given change in the supply than it did twenty years ago.[1] Changes of this kind however, usually come about gradually and the trend can be noted before a marked change occurs.

The time unit used in measuring the effect of the various factors affecting price has varied with the nature of the commodity. With annual crops which can be stored for a year or more, the year has

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[1] Associate in Agricultural Economics.

been the usual unit of time used. Thus in the study of oat prices[4] the total production in the United States in one year plus carry-over was taken as the supply, and the price used was the average price at Chicago for the crop year. The study of potato prices[8] was based on the average price at St. Paul from September to May inclusive, and the supply was the production of the twenty-seven late potato states. As more complete and accurate data become available over a longer time on shipments, storage, and movements into consumption, it may become possible to estimate future prices more accurately for specific periods within the year. Haas and Ezekiel's study of hog prices[2] was based on the month as the unit of time. Hedden[3] in his study of watermelon prices used the day as the unit.

In the study of watermelon prices at Los Angeles it seemed advisable, because of conditions which are described in the following two paragraphs, to take the week as the unit of time.

Most commodities pass through several hands in going from producer to consumer. The price the consumer can be induced to pay for the commodity sets the final limit which any middleman can pay in the long run. Since some time must elapse between the time a commodity is sold to the jobber and the time it is finally sold to the consumer, the middleman must continually make estimates of the prices which the consumers will be willing to pay for a given supply, in order to decide on the price which he (the middleman) can pay and still maintain a necessary margin.

It is evident that the estimates of the middleman will not always be correct, and besides, the producer or shipper also is a party in the bargaining and does what he can to get a favorable return for himself. The price paid for any one lot of watermelons, or the representative price for a day, is probably seldom correctly proportioned to the price which the consumer is willing to pay for the quantity available on that day. The representative price for a week is more likely to be in correct proportion to the price which the consumer is willing to pay.

DATA AVAILABLE

The daily market reports of the U. S. Bureau of Agricultural Economics are available for the Los Angeles market since 1922. These give the daily arrivals, cars on track, and prices of the important fruits and vegetables. Weekly averages of the data on watermelons, cantaloupes, and all other fruits given consistently for the six-year period are shown in table 1. Data on average maximum

temperature lagged three days are also shown. It is often assumed that cantaloupes and other fruits affect the watermelon prices to some extent. These assumptions have been tested mathematically and the results are shown in the following pages.

ANALYSIS OF DATA

The correlation between the supply of watermelons, as measured by the carlots on track (B) and the price (X), has been discussed in Bulletin 449[a] of this station. The gross or simple correlation between these two factors is — 0.8455, which indicates that approximately 71 per cent of the variations in watermelon prices can be accounted for by variations in the supply, leaving 29 per cent to be accounted for by other factors.

Carlot arrivals of watermelons (A) also are fairly closely correlated with prices (X), the gross correlation index being — 0.6604 (see table 2). The net effect of arrivals on prices, however, is not so marked, since carlot arrivals and cars on track are closely associated, as shown by the correlation coefficient of + 0.7405.

One would logically expect the temperature (C) to be positively correlated with watermelon prices, since the demand for watermelons is increased as the temperature goes up. The gross correlation of average maximum daily temperature (lagged 3 days) and of watermelon prices for the weekly periods shown in table 1 is — 0.0583. The negative correlation is due to the fact that temperatures usually go up toward the end of the season while prices decline. When corrections are made for the normal seasonal decline in the price of watermelons, the net effect of a rise in temperature is to raise the price slightly.

Corrections are frequently made for seasonal variations in prices of watermelons first, and the corrected, or adjusted, prices then correlated with the other factors affecting prices. In this study indexes of seasonal variations were calculated and included in the multiple-correlation analysis with the four other factors mentioned above. The index for each week was calculated by taking the arithmetic mean of the average price of the week for each of the six years covered in this investigation.

The gross correlation of the seasonal indexes of prices (D) and the price (X) is + 0.5600. The multiple correlation of these four factors with price (X) was calculated; this gave a multiple-correlation index of 0.861. The residuals obtained from estimates based on the

TABLE 1

RELATION OF LOS ANGELES PRICES FOR WATERMELONS TO WATERMELON CARLOT ARRIVALS AND CARLOTS ON TRACK, TEMPERATURE, SEASONAL INDEX OF PRICES, CANTALOUPE CARLOT ARRIVALS AND CARLOTS ON TRACK, AND IMPORTANT FRUIT CARLOT ARRIVALS AND CARLOTS ON TRACK, 1922-1927

Number of period	Date, period ending	WATERMELONS Average number of carlots each day — Arrivals	WATERMELONS Average number of carlots each day — On track	Maximum temperature in degrees Fahrenheit (3-day lag)	Seasonal index of prices	WATERMELONS Actual price, cents per pound	WATERMELONS Estimated price, cents per pound	CANTALOUPES Average number of carlots each day — Arrivals	CANTALOUPES Average number of carlots each day — On track	IMPORTANT FRUITS Average number of carlots each day — Arrivals	IMPORTANT FRUITS Average number of carlots each day — On track
		A	B	C	D	X	X̄	E	F	G	H
	1922										
1	June 21	6	7	80	276	4 01	3 43	18	5	1.7	0
2	28	26	14	86	223	3.05	3 09	37	17	5.5	4.4
3	July 5	52	54	77	161	1.94	1 80	61	62	4.5	6.0
4	12	45	57	77	139	1 35	1.76	26	51	4.3	2.4
5	19	22	42	81	150	1.59	2 36	14	23	1.4	1.6
6	26	39	45	79	137	1.79	1 74	6	13	12.6	2.6
7	Aug. 2	13	35	80	159	1.75	2.15	9	11	10.6	10.4
8	9	16	10	80	167	2 06	2.58	3	8	6.8	10.0
	1923										
9	June 14	8	8	71	276	3.19	2 77	13	16	2 2	12.0
10	21	28	87	71	223	2.05	1.65	16	24	2.4	5.8
11	28	32	97	70	161	1.63	1.33	24	39	5.4	10.6
12	July 5	43	137	80	139	1.62	1.29	29	35	4.4	7.6
13	12	27	98	75	150	1.64	1.54	12	29	2.6	5.2
14	19	31	83	76	137	2.04	1.53	5	14	6.6	12.0
15	26	20	51	82	159	2.81	2.00	2	3	11.8	28.6
16	Aug. 2	13	44	83	167	2.89	2.22	3	3	10.8	27.6
	1924										
17	June 14	12	22	73	276	2.70	2.71	18	25	1.4	2.8
18	21	21	37	81	223	3.00	2.98	37	52	5.0	6.8
19	28	28	45	76	161	2.23	1.96	21	45	5.4	11.2
20	July 5	35	56	83	139	1.81	2.09	27	74	4.2	4.2
21	12	38	116	78	150	1.83	1.41	13	26	4.6	4.6
22	19	43	109	79	137	1.66	1.41	17	43	5.8	18.4
23	26	27	125	75	159	1.18	1.23	2	10	9.6	12.8
24	Aug. 2	32	65	82	167	1.17	1.60	1	2	20.0	33.2

	Date										
	1925										
25	June 7	9	26	68	276	2.66	2.42	25	32	1.0	1.4
26	14	24	34	74	223	2.21	2.19	26	33	5.4	6.0
27	21	22	72	73	161	1.51	1.53	24	35	10.0	14.6
28	28	36	63	80	139	1.51	1.69	15	24	9.8	12.6
29	July 5	64	107	84	150	2.03	1.51	35	63	5.8	2.8
30	12	48	131	76	137	1.01	0.94	15	44	19.6	13.6
31	19	36	105	88	159	2.04	1.65	6	14	13.0	16.6
32	26	29	66	83	167	1.92	1.57	7	7	22.4	15.8
	1926										
33	June 7	27	37	76	276	1.97	2.38	25	27	6.2	7.8
34	14	41	92	71	223	1.12	1.38	20	29	9.8	16.0
35	21	47	171	73	161	0.82	0.82	13	19	14.6	25.4
36	28	52	168	76	139	0.76	0.93	7	11	9.0	19.4
37	July 5	63	182	82	150	0.80	0.96	3	11	9.4	12.0
38	12	47	144	75	137	0.66	0.87	1	3	19.8	25.4
39	19	35	177	78	159	0.72	0.76	2	1	26.6	40.6
40	26	53	155	84	167	1.03	0.75	2	2	39.2	60.4
	1927										
41	June 14	10	20	71	276	2.05	2.69	35	26	0	0
42	21	17	39	74	223	1.94	2.23	32	37	4.0	5.6
43	28	32	63	77	161	1.54	1.63	41	50	12.0	24.0
44	July 5	43	132	77	139	1.27	1.17	39	67	8.4	20.4
45	12	39	141	86	150	1.09	1.46	34	41	5.4	16.2
46	19	31	142	80	137	1.07	1.08	18	49	16.4	18.6
47	26	44	144	88	159	1.02	1.22	7	15	18.8	27.6
48	Aug. 2	29	137	84	167	0.94	1.11	6	5	24.8	53.8

Columns A, B, X, E, F, G, and H compiled from Harris, Homer A. Market News Service U. S. Bur. Agr. Econ. Daily Market Reports, Los Angeles, Calif., current issues.

Column C compiled from U. S. Weather Bureau Climatological Data—California Section, current issues.

Carlot arrivals represent arrivals since 7:00 a.m. of previous day.

Carlots on track represent morning's count at 7:00 a.m. and includes new arrivals as well as cars partly unloaded.

Prices based on actual prices obtained between 7:00 and 9:00 a.m. by Los Angeles receivers and wholesalers from their sales to jobbers. Where prices were quoted in dollars per carlot, they were converted to cents per pound on the basis of 12 tons to each carlot.†

Column $\bar{X}$ estimated prices based on the regression equation: $\log \bar{X} = -0.3558 - 0.00136A - 0.002305B + 0.00305C + 0.00039C + 0.00063D - 0.00066G$.

Important fruits include apricots, peaches, pears, plums and miscellaneous melons.

multiple regression equation including the four independent factors above, *A*, *B*, *C*, and *D*, were then correlated with cantaloupe carlot arrivals (*E*), carlots of cantaloupes on track (*F*), and carlot arrivals of important fruits (*G*). The residuals gave a correlation index of — 0.37 with factor *G*, which indicated that arrivals of important fruits had an independent effect on watermelon prices of sufficient importance to include with the other four independent factors. The gross correlation with price was — 0.5973. The multiple-correlation index was raised from 0.8610 to 0.8896 by including the effect of arrivals of important fruits (*G*) with factors *A*, *B*, *C*, and *D*.

ACTUAL AND ESTIMATED PRICES FOR WATERMELONS ON THE LOS ANGELES MARKET, 1922–1927
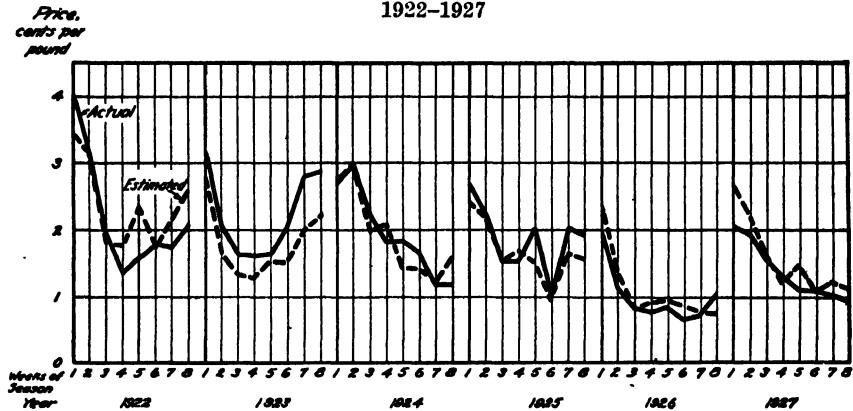
Fig. 1. The estimated prices are based on the average net relationship that prevailed during the entire period, between actual prices and carlot arrivals of watermelons, carlots of watermelons on track, maximum temperature, time of the season, and carlot arrivals of important fruits.

(Data from table 1.)

Having obtained all of the gross correlations between the various factors described in the preceding pages they can be used in obtaining, first the partial regression coefficients, second the coefficients of determination, third the multiple-correlation index, and fourth the regression equation. The method followed is described in detail by Wallace and Snedecor.[7]

MULTIPLE CORRELATION INDEX AND REGRESSION EQUATION

The combined effect of the first five factors, carlot arrivals *A*, carlots on track *B*, temperature *C*, the seasonal index *D*, and carlot arrivals of important fruits *G*, on the price of watermelons *X* at Los Angeles is shown by the multiple correlation index ^{P}log *X.ABCDG*,

which is equal to 0.8896. This takes into account all of the inter-correlations between the independent factors. A correlation index of 0.8896 indicates that approximately 79 per cent of the variations in price can be ascribed to the variations in the five factors mentioned above. The net regression equation obtained by the method described by Wallace and Snedecor[8]2 is as follows:

$$\log \overline{X} = -0.3558 - 0.00136A - 0.00206B + 0.00939C + 0.00063D - 0.00686G$$

On the basis of this equation, which expresses the average net relationship of each of the factors A, B, C, D, and G, and prices (X), the estimated prices in column $\overline{X}$, table 1, were obtained. Approximately one-half of the estimated prices for the past six years based on this equation come within 15 per cent of the actual prices. A comparison of the actual and estimated prices is also shown in figure 1. It will be noted that after the third week in 1922 the actual prices were below the estimated, except in the sixth week, while during 1923 all of the actual prices were above the estimated. Again in 1926 the actual prices were generally below the estimated. Deviations of actual prices from estimated prices were probably due to the following factors:

1. The quality of the watermelons may have been below the average in 1922 and 1926 and above the average in 1923, but no statistical measure of quality for this period exists.

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2 The first step consists in obtaining the partial regression coefficients $\beta \log XA$, $\beta \log XB$, $\beta \log XC$ $\beta \log XD$ and $\beta \log XG$, by solving the following:

$$\beta \log XA + {}^rAB\beta \log XB + {}^rAC\beta \log XC + {}^rAD\beta \log XD + {}^rAG\beta \log XG = {}^rA \log X$$
$${}^rAB\beta \log XA + \quad \beta \log XB + {}^rBC\beta \log XC + {}^rBD\beta \log XD + {}^rBG\beta \log XG = {}^rB \log X$$
$${}^rAC\beta \log XA + {}^rBC\beta \log XB + \quad \beta \log XC + {}^rCD\beta \log XD + {}^rCG\beta \log XG = {}^rC \log X$$
$${}^rAD\beta \log XA + {}^rBD\beta \log XB + {}^rCD\beta \log XC + \quad \beta \log XD + {}^rDG\beta \log XG = {}^rD \log X$$
$${}^rAG\beta \log XA + {}^rBG\beta \log XB + {}^rCG\beta \log XC + {}^rDG\beta \log XD + \quad \beta \log XG = {}^rG \log X$$

The solution of these equations gave the following values:

$$\beta \log XA = -0.1034$$
$$\beta \log XB = -0.5671$$
$$\beta \log XC = +0.2465$$
$$\beta \log XD = +0.1546$$
$$\beta \log XG = -0.2868$$

These values, the means, and standard deviations were substituted in the general equation:

$$\log \overline{X} = M_x + \beta \log XA \cdot \frac{\sigma \log X}{\sigma A}(A - M_A) + \beta \log XB \cdot \frac{\sigma \log X}{\sigma B}(B - M_B)$$

$$+ \beta \log XC \cdot \frac{\sigma \log X}{\sigma C}(C - M_C) + \beta \log XD \cdot \frac{\sigma \log X}{\sigma D}(D - M_D)$$

$$+ \beta \log XG \cdot \frac{\sigma \log X}{\sigma G}(G - M_G)$$

Substituting the values in the above gives:

$$\log \overline{X} = 0.2089 + \left(-0.1034 \times \frac{0.18397}{14.01852}\right)(A - 31.9792)$$

$$+ \left(-0.5671 \times \frac{0.18397}{50.6040}\right)(B - 83.1666) + \left(0.2465 \times \frac{0.18397}{4.82895}\right)(C - 78.1875)$$

$$+ \left(0.1546 \times \frac{0.18397}{45.2296}\right)(D - 176.50) + \left(-0.2868 \times \frac{0.18397}{7.6058}\right)(G - 9.6042)$$

This reduces to

$$\log \overline{X} = -0.3558 - 0.00136A - 0.00206B + 0.00939C + 0.00063D - 0.00686G$$

2. The fluctuations of· actual prices above and below the estimated in 1924 and 1925 suggest the possibility of alternating periods of over and under estimates lasting one or two weeks, in which the dealers misjudged the consumers' demand.

3. Some of the factors that have affected watermelon prices may have been only of a temporary nature which would be impossible to measure accurately.

4. It is difficult to express in one figure a representative price for sales of one day or week, hence the actual prices shown in table 1 may contain errors.

5. The increase in shipments by truck in recent years has made the data on supply somewhat inaccurate.

6. The general price level from 1922 to 1927, according to the Bureau of Labor Statistics index number of all commodities varied from 163 in July, 1925 to 147 in July, 1927. This variation might be expected to account for some of the residuals in prices, but the correlation between the index numbers and residuals of prices was insignificant. Possibly the Bureau of Labor Statistics index number does not represent accurately changes in the price level at Los Angeles.

The above factors undoubtedly explain most of the deviations of actual from estimated prices. Other factors and limitations in the statistical methods must account for the remaining residuals.

CORRELATION OF OTHER FACTORS WITH WATERMELON PRICES

Table 2 also shows the gross correlations of cantaloupe arrivals (E) and carlots of cantaloupes on track (F), and carlots of important fruits on track (H) with watermelon prices. It seems reasonable to expect that large supplies of cantaloupes or other fruits would tend to depress watermelon prices. However, the gross correlation index between carlot arrivals of cantaloupes and watermelon prices is + 0.3083 which indicates that for the 48 weeks shown in table 1 there has been a slight tendency for the opposite relationship to prevail.

The explanation for this contradiction between what one might expect and what one finds lies in the differences in the seasonal move-ment of cantaloupes and watermelons. Cantaloupe arrivals are often at their peak about the second or third week of the watermelon season, whereas watermelon arrivals usually reach their peak in the fourth, fifth, or sixth weeks. From that time on there is usually a decline in arrivals which often occurs at the same time as the seasonal decline

in watermelon prices. When the effect of the first four factors *A, B, C,* and *D* (table 1) on prices was taken into account, and the residuals of prices (the differences between the logarithms of actual and estimated prices) correlated with carlot arrivals of cantaloupes, the correlation index became + 0.1606, which is of no practical significance so far as showing an independent effect on watermelon prices is concerned.

TABLE 2

GROSS CORRELATIONS OF WATERMELON PRICES AND EIGHT FACTORS, AND
INTERCORRELATIONS OF *A, B, C, D,* AND *G*

Factors correlated	Watermelons		Temperature	Seasonal index of prices	Cantaloupes		Important fruits	
	Carlot arrivals	Carlots on track			Carlot arrivals	Carlots on track	Carlot arrivals	Carlots on track
	A	*B*	*C*	*D*	*E*	*F*	*G*	*H*
log X (Watermelon prices)	−0 6604	−0.8455	−0.0583	+0 5600	0 3038	0.1063	−0.5973	−0.5383
A	....	0.7405	0.2718	−0.6310	... .	... .	0.3713	... .
B	... ...	....	0.1824	−0.5865	... .	.. ..	0 5443	... ...
C	....	.. ..	....	−0.4075	... .. .		..... .	0.3845	... .
D	... ...	... .	. ...	....	....	....	−0.3769	....

The carlots of important fruits on track (*H*) gives a gross correlation index of − 0.5383 with prices, but when the effect of factors *A, B, C, D,* and *G* is taken account of the correlation with the price residuals becomes + 0.0349.

The efforts to improve the accuracy of the watermelon price estimates by including factors *E, F,* and *H* (tables 1 and 2) proved fruitless. The most accurate method discovered so far is on the basis of the five factors *A, B, C, D,* and *G.*

IMPORTANCE OF THE INDIVIDUAL FACTORS

The coefficients of determination[3] give at least a rough measure of the relative importance of the different factors affecting prices. Expressed in percentages they are as follows:

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[3] The coefficient of determination is the product of the partial regression coefficient (see footnote 2, p. 311) and the corresponding gross correlation index shown in table 2. The sum of the coefficients of determination equals the square of the multiple correlation index.

Thus $P^2 = \beta \log XA \cdot {}^rA \log X + \beta \log XB \cdot {}^rB \log X + \beta \log XC \cdot {}^rC \log X + \beta \log XD \cdot {}^rD \log X + \beta \log XG \cdot {}^rG \log X$

Substituting

$P^2 = (-0.1034 \times -0.6604) + (-0.5671 \times -0.8455) + (+0.2465 \times -0.0583) + (0.1546 \times +0.5600) + (-0.2968 \times -0.5973) = +0.0683 + 0.4795 - 0.0144 + 0.0866 + 0.1713 = +0.7913$

$P = \sqrt{0.7913} = 0.8896$

A—Average daily carlot arrivals of watermelons .... + 6.83 per cent
B—Average carlots of watermelons on track .... + 47.95 " "
C—Average of maximum temperatures (3-day lag) ...... — 1.44 " "
D—Seasonal variation in price .... + 8.66 " "
G—Average daily carlot arrivals of important fruits .... + 17.13 " "

Total 79.13

The algebraic sum of the coefficients of determination is 79.13, which indicates that approximately 79 per cent of the variation in prices are accounted for by the variation in these five factors. The negative coefficient of determination for temperature shows that its effect is in the opposite direction from that of the other factors. The most important factor affecting prices is the number of carlots of watermelons on track. Next in importance is the carlot arrivals of important fruits, third, the seasonal factor, and fourth, the carlot arrivals of watermelons.

The square root of the sum of the coefficients of determination gives the multiple correlation index $P_{\log X.ABCDG} = 0.8896$.

TABLE 3

AVERAGE NET EFFECT OF CARLOT ARRIVALS OF WATERMELONS (A)
ON PRICE (X)

Carlot arrivals	Price* in cents per pound
A	X
0	1.79
10	1.73
20	1.68
30	1.63
40	1.58
50	1.53
60	1.48
70	1.44

* Based on the regression equation $\underline{Log\ \overline{X} = 0.25238 - 0.00136A}$.

EFFECT OF THE INDIVIDUAL FACTORS ON WATERMELON PRICES

Carlot Arrivals of Watermelons.—The regression equation showing the average net effects of the carlot arrivals (A), carlots on track (B), temperature (C), seasonal indexes (D), and carlot arrivals of important fruits (G), on price (X) is as follows:

$$\log \overline{X} = -\,0.3558 - 0.00136A - 0.00206B + 0.00939C + 00063D$$
$$-\,0.00686G$$

Now substituting the means of B, C, D, and G in this equation and varying A, the net effect of variations in A are obtained; these are

shown in table 3 and figure 2. When the number of carlots increased
from ten to twenty, the price decreased on an average from 1.73 cents
to 1.68 cents a pound, approximately 3 per cent. An increase in
carlot arrivals from ten to seventy carlots decreased the price 17
per cent.

AVERAGE NET EFFECT OF CARLOT ARRIVALS OF WATERMELONS UPON PRICES
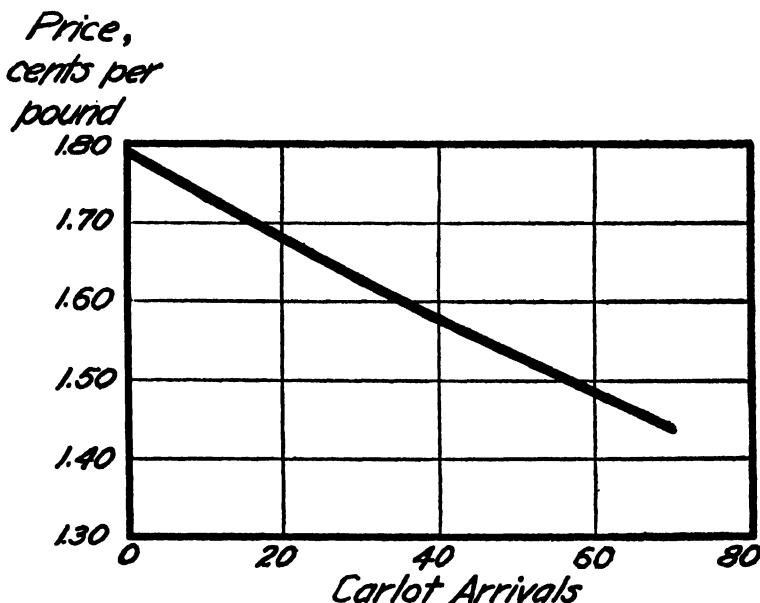
Fig. 2. The average net effect of each increase of 10 carlots was to decrease
the price approximately 3 per cent.   (Data from table 3.)

Carlots of Watermelons on Track.—The average net effect on price
of carlots on track is obtained in the same way as for carlot arrivals.
The results are shown in table 4 and figure 3. An increase from
twenty carlots to forty carlots on track was accompanied (other fac-
tors being at an average) by a decrease in price from 2.18 cents to
1.99 cents or approximately 9.0 per cent.

Maximum Temperature.—The average net effect of maximum tem-
perature, lagged three days, on watermelon prices is shown in table 5
and figure 4. An increase of four degrees Fahrenheit in temperature,
other factors remaining at an average, resulted in an average increase
of 9.0 per cent in price. Thus with the temperature at 68° Fahren-
heit (see table 5), with other factors at an average, the price was
1.29 cents a pound. An increase in temperature to 72° Fahrenheit
raised the price to 1.42 cents, approximately a 9.0 per cent increase.

AVERAGE NET EFFECT OF WATERMELON CARLOTS ON TRACK UPON PRICES
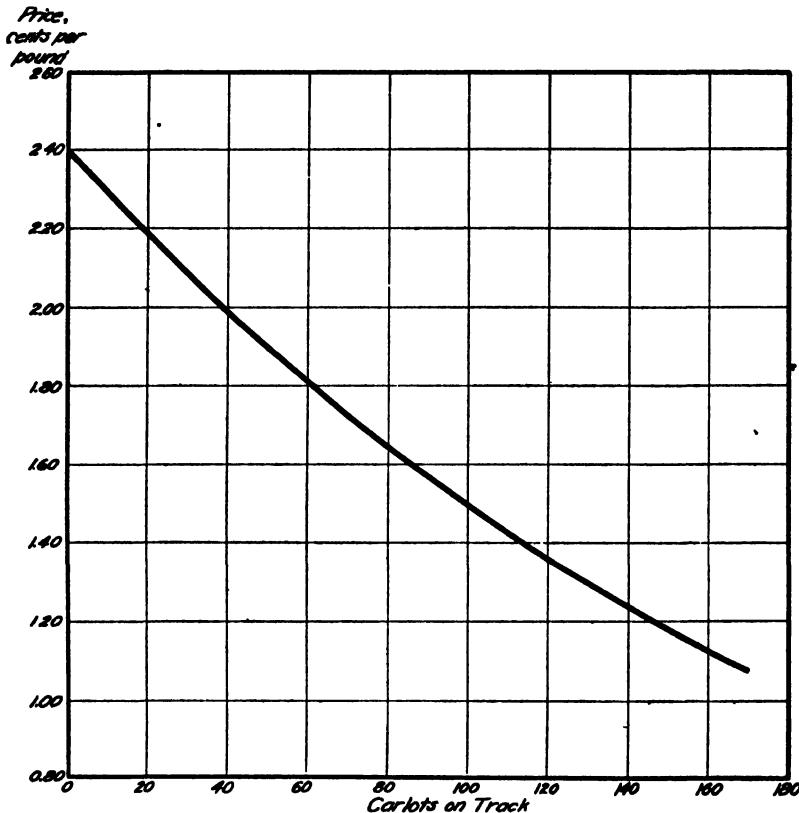
Fig. 3. Each increase of 10 carlots on track had the average net effect of reducing the price approximately 4.5 per cent.   (Data from table 4.)

TABLE 4

AVERAGE NET EFFECT OF CARLOTS ON TRACK OF WATERMELONS (B)

ON PRICE (X)

Carlots on track	Price* in cents per pound
B	X
0	2 40
20	2 18
40	1 99
60	1 81
80	1 64
100	1 49
120	1 36
140	1 24
170	1 07

* Based on the regression equation $Log \ \overline{X} = 0.38021 - 0.00206B$.

AVERAGE NET EFFECT OF TEMPERATURE (LAGGED THREE DAYS) UPON
WATERMELON PRICES
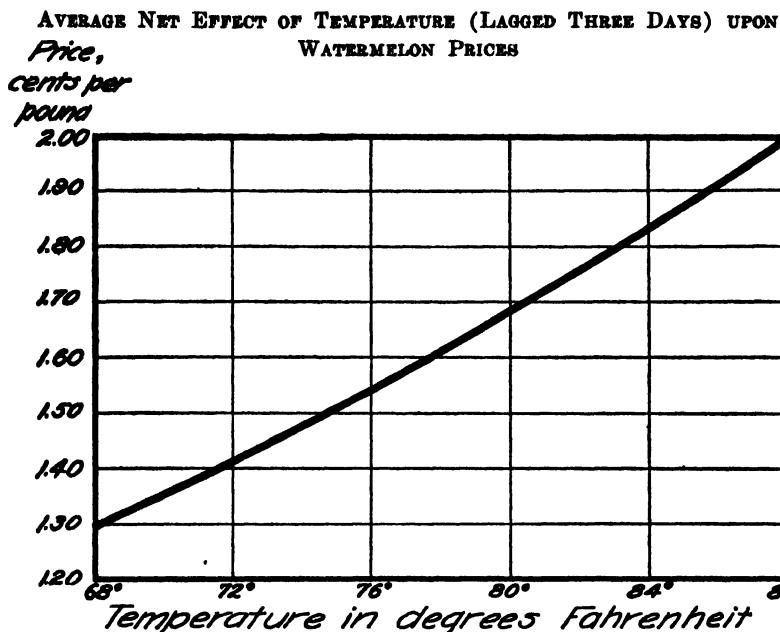
Fig. 4. With other factors held constant, the average net effect of an increase of four degrees Fahrenheit in temperature was to raise prices approximately 9.0 per cent.   (Data from table 5)

TABLE 5

AVERAGE NET EFFECT OF TEMPERATURE (LAGGED 3 DAYS) (C)
ON PRICE OF WATERMELONS (X)

Temperature in degrees Fahrenheit	Price* in cents per pound
C	X
68	1 29
72	1 42
76	1 54
80	1 68
84	1 83
88	2 00

* Based on the regression equation $Log\ \overline{X} = -0\ 52529 + 0\ 00939C$

Time of the Season.—The average net effect of the time of the season is shown in table 6 and figure 5. Holding the other factors at an average, the price of watermelons the second week averaged 1.73 cents a pound compared with 1.87 cents for the first week (see table 6 and figure 5). In other words the net effect of the advance of the watermelon season from the first to the second week was to lower the price 7.4 per cent. The low point of the season was reached in the sixth week, after which there was a slight recovery.

AVERAGE NET EFFECT OF TIME OF SEASON UPON WATERMELON PRICES
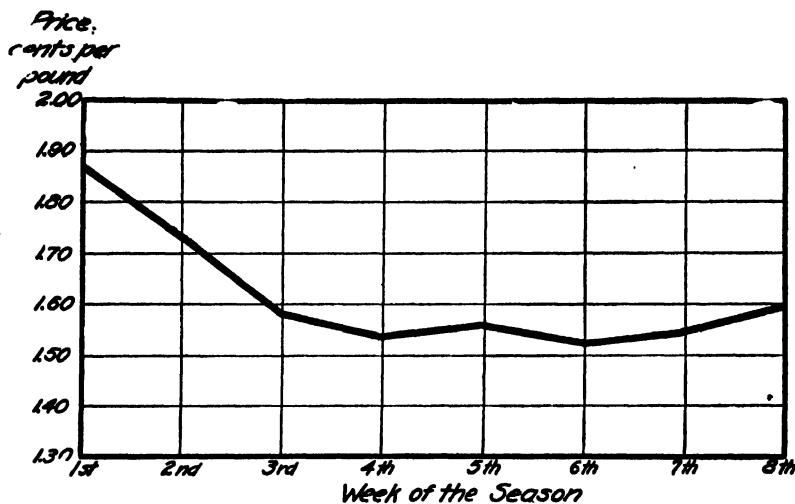
Fig. 5.—The average net effect of the time of the season on prices was to cause them to decline until after the fourth week. Some recovery occurred in the fifth, seventh, and eighth weeks.

(Data from table 6.)

TABLE 6

AVERAGE NET EFFECT OF SEASONAL INDEX (D) ON PRICE (X)

Week of season	Seasonal index	Price* in cents per pound
	D	X
1	276	1.87
2	223	1.73
3	161	1.58
4	139	1.53
5	150	1.56
6	137	1.53
7	159	1.55
8	167	1.60

* Based on the regression equation $Log \overline{X} = 0.09769 + 0.00063D$.

Carlot Arrivals of Important Fruits.—Table 7 and figure 6 show the net effect of carlot arrivals of important fruits on watermelon prices. An increase in arrivals of ten carlots of the important fruits (apricots, peaches, pears, plums, and miscellaneous melons, the fruits for which records are available for each of the years 1922 to 1927) caused a decrease of 14.6 per cent in price. For example, the increase in arrivals from ten carlots to twenty carlots brought an average decrease in price from 1.61 to 1.37 cents a pound, or 14.6 per cent.

AVERAGE NET EFFECT OF CARLOT ARRIVALS OF IMPORTANT FRUITS UPON
PRICES OF WATERMELONS
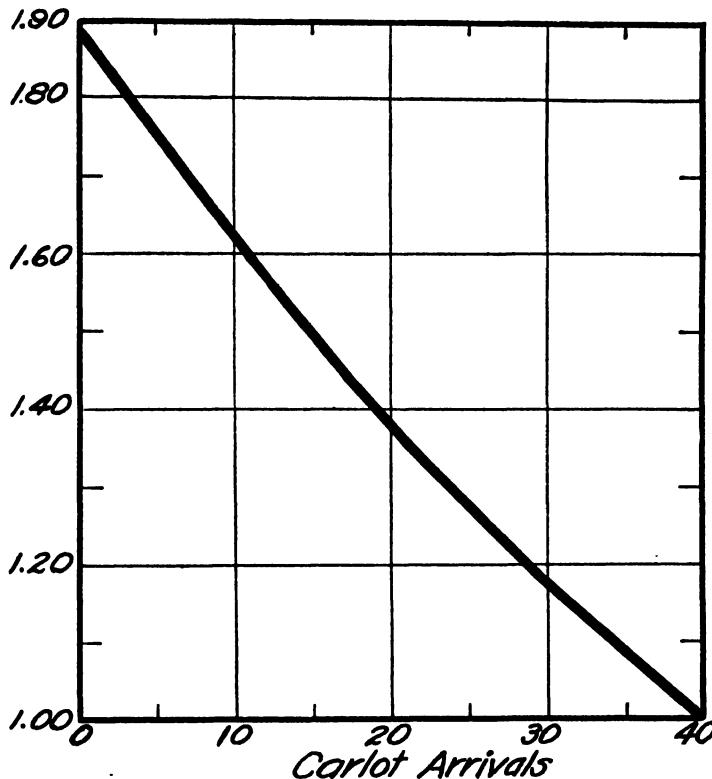
Fig. 6.   An increase of 10 carlots in arrivals of important fruits (other
factors remaining equal) brought an average decrease in price of 14.6 per cent.
(Data from table 7.)

TABLE 7

AVERAGE NET EFFECT OF CARLOT ARRIVALS OF IMPORTANT FRUITS* (G)
ON PRICE X

Carlot arrivals	Price† in cents per pound
G	X
0	1.88
5	1.74
10	1.61
20	1.37
30	1.17
40	1.00

* Important fruits include apricots, peaches, pears, miscellaneous melons, and plums.
† Based on the regression equation Log $\bar{X}$ = 0.27477 − 0.00686G.

HOW TO USE THE RESULTS OF THESE STATISTICAL ANALYSES

Every buyer and seller must make estimates of the prices that will move a given supply of a commodity under a given set of conditions. A large part of the success of anyone engaged in buying and selling depends upon the accuracy of his estimates of the prices that will equate supply and demand. The only basis for making these estimates is past experience. The estimates may be based on some mental calculations of figures that left their impress on the mind, or it may be based on careful analysis of statistical data that have been kept over a long time plus any other knowledge of a non-statistical nature that every business man accumulates. In either case the assumption is made that the factors considered will continue to have the same effect on prices in the future that they have had in the past. If this did not generally hold true we would have no basis for estimating the future by any method.

The results obtained on factors affecting watermelon prices at Los Angeles should not be used without an understanding of their limitations and an appreciation of the need of an intimate knowledge of the business in addition to the quantitative relationships shown in the equation on pages 311 and 314. Estimating the most probable price on the basis of carlots on track has been explained in a previous bulletin.[6]

Estimating the most probable price on the basis of the factors used in the equation can be illustrated by estimating the price for a certain time in 1928 assuming a definite set of conditions. The price estimates shown in table 1 are based upon the relationship of average prices by weeks, and averages for the same weeks of the various factors affecting prices, except temperature, which is for periods three days earlier.

Hence in order to estimate, say on a Monday morning, the price for that week ending Friday, it would be necessary to first estimate the average daily arrivals of watermelons and important fruits and the carlots of watermelons on track for the rest of the week.

This would mean estimating prices on the basis of supply estimated so far ahead that most dealers would doubtless prefer to estimate the prices directly rather than in the round-about way. However, if receipts and cars on track were estimated two days in advance and an average worked out for the week ending on Wednesday morning, a very close adjustment could be made for expected changes in

arrivals and carlots on track for the next two days. The correlation with temperature is based on a three-day lag of temperature so that the average temperature up to Sunday would give data corresponding to the other data for the period ending the next Wednesday. The seasonal index of prices is selected according to the week of the season in which the period in question happens to fall.

Let us assume now that the Monday comes in the third week of the season, and that the various adjustments give the following values:

> A—Average carlots arriving daily of watermelons for week ending
>   on Wednesday .... $=32$
> B—Average carlots on track of watermelons each day for week end-
>   ing on Wednesday .... $=63$
> C—Average maximum temperature for week ending on Sunday .... $=77$
> D—Seasonal index of price (third week) .... $=161$
> G—Average carlots of important fruits arriving daily for week end-
>   ing on Wednesday .... $=12$

Substituting these values in the equation

$\log \overline{X} = -0.3558 - 0.00136A - 0.00206B + 0.00939C + 0.00063D - 0.00686G$

gives

$\log \overline{X} = -0.3558 - 0.00136 \times 32 - 0.00206 \times 63 + 0.00939 \times 77$
$\qquad + 0.00063 \times 161 - 0.00686 \times 12$
$\qquad = -0.3558 - 0.04352 - 0.12978 + 0.72303 + 0.10143 - 0.08232$

$\log \overline{X} = 0.21304$

Looking up the anti-log of 0.21304 we obtain $\overline{X} = 1.63$ cents—the estimated price per pound for that week. Estimates such as the above should be of value to shippers in deciding on the number of carlots that can be shipped to Los Angeles before losses are likely to be sustained, and to buyers and sellers in deciding on what price is justified on the basis of the conditions prevailing at a particular time.

SUMMARY

A statistical analysis of the factors affecting average weekly prices of watermelons at Los Angeles indicates that the most important factors, in the order named are: carlots of watermelons on track, car-lot arrivals of important fruits, time of the season, carlot arrivals of watermelons, and maximum temperature lagged three days. Weekly averages of supplies, arrivals, and temperatures were obtained for the first eight weeks of each season from 1922 to 1927, and seasonal indexes of prices were calculated. Variations in these five factors accounted for 79 per cent of the variations in price.

The average relationship of these factors and watermelon prices is expressed by the equation

$$\log \overline{X} = -0.3558 - 0.00136A - 0.00206B + 0.00939C + 0.00063D - 0.00686G$$

which can be used in estimating future prices $(\overline{X})$ when carlots on track (A), carlot arrivals (B), maximum temperature lagged three days (C), the seasonal indexes (D), and carlot arrivals of important fruits (G) are known or can be closely estimated. Applying this equation over the past six years approximately one-half of the estimated prices come within 15 per cent of the actual prices. Some of the variations of estimated from actual prices are undoubtedly due to the fact that shippers and jobbers cannot estimate the consumer's demand accurately, and hence actual prices may sometimes be above and sometimes below the price which would equate supply and demand. The quality of the watermelons—a factor on which no statistical data are available—variations in truck shipments, and the difficulty of obtaining representative prices probably were the most important remaining factors causing variations of actual from estimated prices.

It seemed logical to expect that cantaloupe arrivals and carlots on track would also affect watermelon prices, but practically no net correlation was found to exist between them. The same thing was true of carlots on track of important fruits, after the effect of the other factors, including carlot arrivals of important·fruits, had been taken into consideration.

ACKNOWLEDGMENTS

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FACTORS AFFECTING THE PRICE OF GRAVENSTEIN APPLES AT SEBASTOPOL

EMIL RAUCHENSTEIN[1]

THE PROBLEM

Early in 1927 the Gravenstein apple growers in the Sebastopol district organized partly for the purpose of strengthening their bargaining position with buyers in determining the price which they should receive for their apples. This brings up the problem of estimating, at the beginning of the season, the price which will equate supply and demand under the conditions prevailing that season.

While it is generally known that a large crop usually brings a low price per box, and a small crop usually brings a high price, no schedule has been worked out up to this time, on the basis of the average relationship that has prevailed between supply and price, showing the price which crops of various sizes have brought; and no study has been made of the effect on prices of other factors than the supply of Gravensteins in the Sebastopol district. It is the purpose of this paper to present and analyze the data available on this problem in order to determine the important factors that have affected prices in the past, and to show how the results may be used, as a starting point at least, for estimating a fair price at the beginning of the season under a given set of conditions.

DATA AVAILABLE

Records of Gravenstein apple production in the Sebastopol district and prices received by farmers at the packing plants are available from 1912 to date, and are shown in table 1 and figure 1. Table 1 also gives the all-commodity index number for July of each year, which gives a fairly good measure of the changing value of the dollar. By dividing the price for each year by the July index number of that year and multiplying the quotient by 1.50 the adjusted price

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series, shown in column X, is obtained. This shows the prices
approximately. as they would have been if the dollar had had the
same purchasing power during the whole period as it had during
1927. The prices referred to hereafter in this paper are these
adjusted prices.

The estimates (July 1 and final) of total United States apple
production are also shown in table 1 and the July 1 estimates in
figure 1.

TABLE 1

APPLE PRODUCTION ESTIMATES OF JULY 1 AND FINAL ESTIMATES FOR THE UNITED
STATES, GRAVENSTEIN APPLE PRODUCTION IN THE SEBASTOPOL DISTRICT,
AND GRAVENSTEIN APPLE PRICES, 1912-1927

| Year | United States production | | Gravenstein apple production in Sebastopol district (Thousands of boxes) B | Price of Gravensteins per box to farmers at packing plants† | July index‡ numbers (all commodity) | Price adjusted to 1927 price level X |
	Estimates of July 1 (Millions of bushels) A	Final* Estimates (Millions of bushels)				
1912	228	235	53	$0.54	101	$0.80
1913	209	145	42	0.98	102	1.44
1914	210	253	77	0.46	99	0.71
1915	194	230	80	0.52	102	0.77
1916	219	194	96	0.61	125	0.74
1917	200	167	152	0.93	191	0.74
1918	195	170	194	1.43	200	1.06
1919	156	142	322	1.98	216	1.38
1920	200	224	400	1.73	245	1.05
1921	102	99	367	1.72	144	1.79
1922	190	203	714	0.47	158	0.45
1923	189	203	1,172	0.73	153	0.72
1924	196	172	563	1.15	150	1.15
1925	179	172	126	1.96	163	1.80
1926	208	246	1,134	0.39	153	0.39
1927	137	123	680	1.66	147	1.70

Column A, data obtained from July numbers of Crops and Markets Monthly Supplement and
official series of U. S. Dept. Agr. preceding Crops and Markets.
* From U. S. Dept. Agr. Yearbook 1926: 896, 1927; except for 1927 which is from Crops and Markets
4 (12): 453. 1927.
Column B, data obtained from representative shippers by H. F. Gould and L. W. Fluharty.
† Data obtained by L. W. Fluharty from representative shippers. Average of Fancy 4 and 4½ tier.
‡ Bureau of Labor Statistics index number converted to 1910-1914 base. U. S. Dept. Agr. Supple-
ment to Agriculture Situation June 1927, and current issues.

ANALYSIS OF DATA

General Analysis.—Having eliminated the effect of the changing
value of the dollar on prices, it is possible from a close study of
figure 1 and table 1 to find some evidence of consistent inverse corre-
lation between production and price (adjusted). Thus from 1912
to 1913 there was a decrease in the July 1 estimate of United States

production associated with a considerable increase in the price of Gravenstein apples. Again there was a decrease in production estimates in each of the years 1917, 1918, and 1919 associated with consistent increases in price. The years 1920, 1921, and 1922 are also good examples of this inverse correlation.

COMPARISON OF GRAVENSTEIN APPLE PRICES WITH PRODUCTION IN SEBASTOPOL DISTRICT AND JULY 1 ESTIMATES OF TOTAL UNITED STATES APPLE PRODUCTION
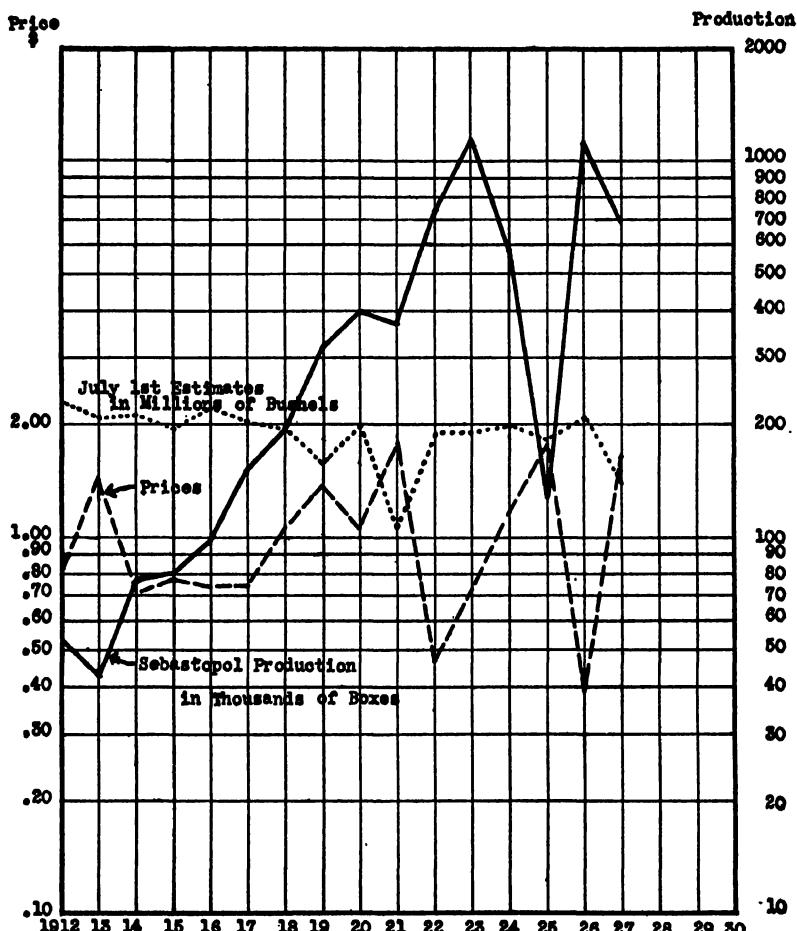
Fig. 1. The inverse correlation between Gravenstein apple production and price in the Sebastopol district is not at all marked until about 1919. Since then it has been noticeable. July 1 estimates of United States production also shows an inverse correlation with Gravenstein prices.

(Data from table 1.)

The inverse correlation between Gravenstein apple production in the Sebastopol district and prices from 1912 to 1918 is not evident, except for the first two years. When one notes the low production of Gravenstein apples—no year above 100,000 boxes during the first five years covered by this study—it becomes evident that they were such a small factor in the market during these years that they would not affect prices to an appreciable extent. The rapid increase in production after 1916 is shown by the fact that although up to that time production was never above 100,000 boxes a year, eight years later (in 1923) the production exceeded 1,000,000 boxes. During several years since 1920, California apple shipments in July which consist mainly of Gravensteins from the Sebastopol district, amounted to one-third of the total United States apple shipments for that month (see table 3).

Beginning with 1919 there is a noticeable inverse correlation between Gravenstein apple production and price. With the exception of 1923 the price changes from the previous years were in the opposite direction from the production. The amount of change in price, however, is apparently affected by both Gravenstein production in the Sebastopol district and United States production. The drop in price from $1.38 to $1.05 from 1919 to 1920 was probably caused only in part by the increase in Gravensteins from 322,000 to 400,000 boxes, and in part by the increase in United States production (July 1 estimates) of 156,000,000 to 200,000,000 bushels. The increase in price in 1921 to $1.79 again was probably due in part to the slight drop in Gravenstein production, but more largely to the big drop (from 200,000,000 to 102,000,000 bushels) in United States production. From 1918 to 1919 Gravenstein production at Sebastopol went up while United States production went down. The latter factor seemed to exert the greater influence since the price of Gravensteins went up.

Correlation Analysis.[2]—The degree of relationship between two variables can be determined with considerable accuracy by means of the correlation coefficient. The final estimates of apple production in the United States from 1919 to 1927 and prices of Gravensteins show a correlation coefficient of — 0.80. However, if growers are to have any basis for estimating the price which Gravenstein apples should bring, data must be used which become available before the

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[2] The method used in these calculations is described in detail in the publication by Wallace, H. A., and Geo. W. Snedecor. Correlation and machine calculation. Iowa State College of Agr. and Mechanic Arts, Official Publication 23:1–47. 1925.

crop is sold. Since the crop is usually shipped during July and the first part of August, the July 1 estimate of total apple production should give the best indication of the probable effect of production in the country as a whole.

The estimates of July 1 were not as close to the final production during the first years of the period as they were later, although even from 1914 to 1927 the correlation coefficient between them and Gravenstein prices is — 0.77.

A study of the other factor (production of Gravensteins in the Sebastopol district) clearly shows some association with prices during recent years. Some difficulties are involved, however, in evaluating its effect for the whole period, because of the rapid increase in production from less than 100,000 boxes in 1912 to more than 1,000,000 boxes in 1923 and 1926. Disregarding trend in production the correlation coefficient between Gravenstein apple production in the Sebastopol district and price was — 0.2602 for the period 1912 to 1927, and — 0.2616 for the period 1914 to 1927. Obviously these correlation coefficients do not show the true relationship for the later years because of the irregular upward trend in production, and further the effect has probably been increasing relative to the effect of total United States production.[3]

A multiple regression equation based on the period 1914 to 1927 for estimating prices from July 1 estimates of United States production and Gravenstein production in the Sebastopol district would probably overemphasize the effect of the latter.

In order to compare possible changes in the regression equation for various periods the necessary calculations were made based on the relationships between the three factors for the period 1914–1927. The resulting equation was $\bar{X} = 3.3741 - 0.01179A - 0.000402B$, in which $\bar{X}$ represents estimated price in dollars, A, July 1 estimates of United States production, and B, Gravenstein production in the Sebastopol district. The multiple correlation coefficient ($R_{X.AB}$) was equal to 0.8298, the standard error of estimate ($S_{X.AB}$) was 0.256, and the coefficients of determination indicated that approximately 61 per cent of the variations in price were due to United States production and 8 per cent to Sebastopol production of Gravensteins. Price estimates for the period 1914–1927 based on the above equation fall within $0.25 of the actual price ten out of the fourteen years.

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[3] By correlating first differences of logarithms of production and prices a correlation index of — 0.54 is obtained. This method does away with the difficulty of the production trend, but cannot show whether or not the relationship is becoming closer toward the end of the period.

Correlation of Production and Price from 1919 to 1927.—Figure 1 shows that Gravenstein apple production in the Sebastopol district increased very rapidly from 1913 to 1919. Since 1919 the trend upward has been much less pronounced and in one year (1925) the production was below that of 1919. An analysis of the average relationships that have prevailed between production and price during the later period therefore should give results more applicable to the problem of estimating the price that will move the 1928 and 1929 crops, than the results based on the average relationship for the whole period 1912 to 1927, in spite of the fact that only nine years are included in the later period. The correlations for the later period are in line with what one would logically expect. That is, since 1919 the Sebastopol crop of Gravensteins has become a more important factor on the markets in July and August than it was previous to 1919, and therefore its effect on Gravenstein apple prices during the later period has been much more marked than it was for the earlier period. This is brought out by the coefficients of determination, which indicate that for the period 1919 to 1927, 40.7 per cent of the variations from year to year in Gravenstein apple prices were associated with variations in production of Gravensteins in the Sebastopol district, compared with 8.0 per cent for the period 1914 to 1927; and 38.5 per cent were associated with variations in the July 1 estimates of total United States production in the later part of the period, compared with 61.0 per cent for the whole period. .

Table 2 and figure 2 show the actual and estimated prices of Gravenstein apples. The estimated prices are based on the average relationship of actual prices and the production data noted above for the period 1919 to 1927. This period, of course, is too short to constitute a very reliable statistical sample, but the results bear out the logical expectations.

The residuals (actual prices minus estimated) in table 2 are $0.17 or less in six years out of the nine. The extremely low price of 1922 may have been caused in part by the railroad strike at the time Gravensteins were being shipped.[4] In 1925 the actual price was $0.29 above the estimated price. Apparently the extremely short crop (126,000 boxes) in the Sebastopol district exerted more than a proportional upward pull on the price. In 1927 the actual price again exceeded the estimated, this time by $0.30. Possibly the organization of growers in 1927 which quoted a uniform price to buyers may have strengthened their bargaining power to the extent of $0.30 a box.

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[4] This suggestion was made by Mr. G. E. Burlingame, secretary of the Sebastopol Chamber of Commerce.

TABLE 2

ACTUAL AND ESTIMATED PRICES OF GRAVENSTEIN APPLES, 1919–1927

Year	Actual adjusted price X	Estimated price $\overline{X}$	Residuals Z
1919	$1.38	$1.54	$-0.16
1920	1.05	1.11	−0.06
1921	1.79	1.95	−0.16
1922	0.45	0.93	−0.48
1923	0.72	0.55	+0.17
1924	1.15	1.01	+0.14
1925	1.80	1.51	+0.29
1926	0.39	0 43	−0.04
1927	1.70	1.40	+0.30

Column $\overline{X}$ calculated from the equation $\overline{X} = 3.10901 - 0.00832\ A - 0.000839B$.
Residuals Z equal actual minus estimated prices.

ACTUAL AND ESTIMATED FARM PRICES OF GRAVENSTEIN APPLES
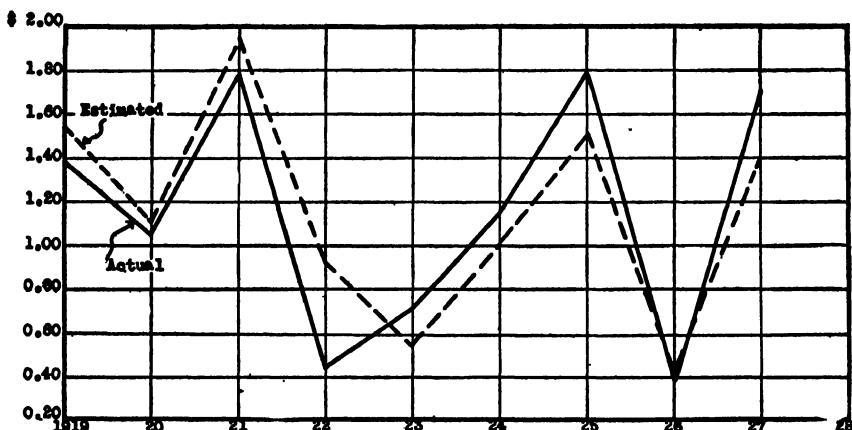
Fig. 2. Estimates of Gravenstein apple prices based on the average relationship between prices, July 1 estimates of United States production, and Gravenstein production in the Sebastopol district come within $0.17 of the actual price six years out of nine.

(Data from table 2.)

Possible Causal Relationships Involved in the Determination of Gravenstein Apple Prices.—The fact that there is a high degree of association between two variables does not prove that one is necessarily the antecedent or cause of the other. In the case of Gravenstein apple production in the Sebastopol district and prices of Gravensteins at Sebastopol, it seems clear that high production would cause low prices (other things remaining the same) and low production

would cause high prices. The correlation coefficient between these two variables from 1919 to 1927 was — 0.749, which may be considered as representing a cause and effect relationship. With July 1 estimates of total United States production and Gravenstein apple prices, the cause and effect relationship is not so self evident. Are buyers influenced by the supply coming onto the markets in July and early August, or by their anticipation of the size of the total crop? Probably both factors affect the price. Table 3 and figure 3

TABLE 3

TOTAL APPLE SHIPMENTS IN THE UNITED STATES AND FROM CALIFORNIA FOR JULY AND AUGUST, AND FARM PRICES OF GRAVENSTEIN APPLES AT SEBASTOPOL, 1919–1927

Year	July shipments		August shipments		Prices (adjusted) to farmers at Sebastopol
	Total United States carlots	California carlots	Total United States carlots	California carlots	
1919	1,347	273	2,712	441	$1.38
1920	1,855	244	3,861	723	1.05
1921	1,207	352	3,384	690	1.79
1922	2,592	220	4,923	998	0.45
1923	3,360	1,290	4,122	984	0.72
1924	2,362	729	3,126	645	1.15
1925	2,895	341	4,330	155	1.80
1926	3,665	1,480	3,131	591	0.39
1927	1,731	289	3,352	841	1.70

Prices from Table 1.
Shipments for 1919–1922 compiled from U. S. Dept. Agr. Statis. Bul. 7:2–5; for 1923–1926 from Monthly Supplement of Crops and Markets 3 (8, 9) 1926; for 1927 from Monthly Supplement of Crops and Markets 4 (8, 9), 1927.

show apple shipments in July and August in the United States, California apple shipments in July, and the price of Gravensteins at Sebastopol. In general, low July shipments were associated with high prices, and high shipments with low prices. The year 1923 seems to be an exception since the price and shipment changes from 1922 are both upward. This may be due to the railroad strike in 1922 (mentioned on p. 330) which seems to have depressed the 1922 price much below normal for the production of that year. Compared with other years than 1922, the price in 1923 seems to be normal considering the quantity shipped. The year 1925 is noteworthy in that price and shipments were both higher than in 1924. Gravenstein prices in 1925 seem to have been affected specifically by the short crop of Gravensteins since shipments for the United States as a whole were relatively high, but shipments from California were very low. The

correlation coefficient between July shipments in the United States and Gravenstein prices for the period 1919 to 1927 was — 0.65, between California apple shipments in July and Gravenstein prices — 0.58, and between July 1 estimates of United States production and Gravenstein prices it was — 0.74.

APPLE SHIPMENTS IN THE UNITED STATES AND CALIFORNIA AND PRICES OF
GRAVENSTEIN APPLES
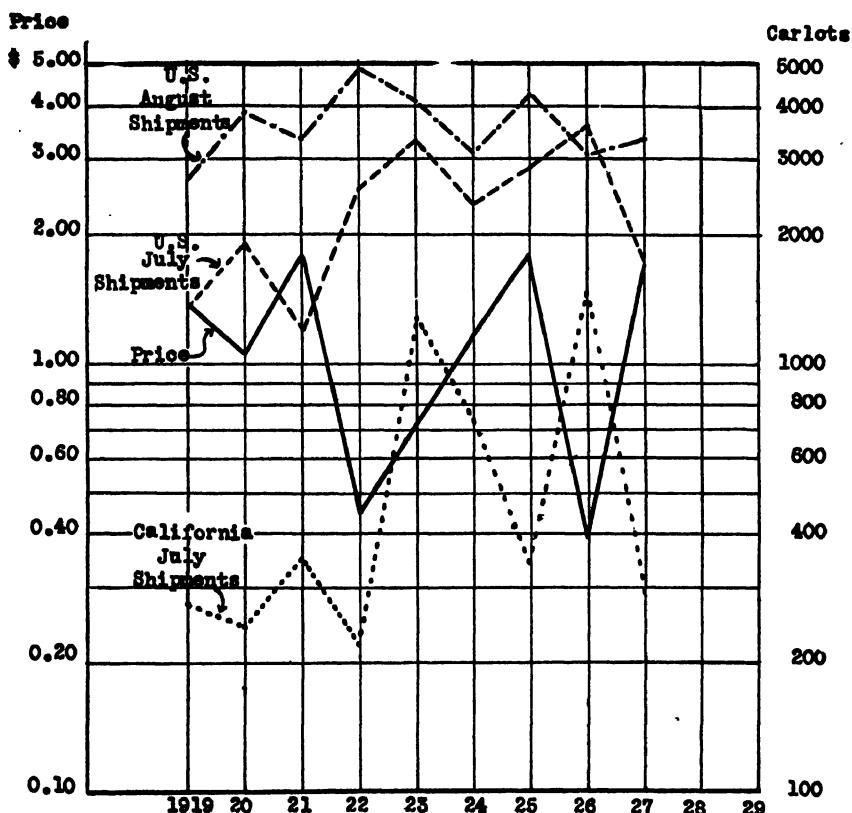
Fig. 3. High apple shipments in the United States in July and low prices for Gravensteins tend to go together. and low shipments go with high prices. Since 1923 California shipments in July and Gravenstein prices show a close inverse correlation.

(Data from table 3.)

August shipments in the United States are not as closely correlated with Gravenstein apple prices as are the July shipments. The correlation coefficient is — 0.3819. This smaller correlation is to be expected because the bulk of the Gravenstein apple crop is usually marketed in July.

There is a fairly close correlation between July shipments in the United States from 1919 to 1927 and July 1 estimates of United States production. The correlation coefficient is — 0.73. The correlation coefficient between July shipments and final estimates of production is also — 0.73. Probably one reason why the correlation between July apple shipments in the United States and Gravenstein apple prices is not closer than the correlation of July 1 estimates of United States production and prices is that July shipments vary from year to year, partly as the result of variations in the size of the total crop, and partly as the result of the time of ripening. If accurate data could be obtained as to the total quantities of apples coming onto the markets during the period in which Gravenstein apples are marketed, they would probably show a higher correlation coefficient with price than that obtained between July 1 estimates and price.

The practical conclusion to be drawn from these correlation studies is that the July 1 estimates of United States production are the best figures to use in estimating the effect of the country's total apple production on the price of Gravenstein apples because they probably indicate fairly accurately the competition which Gravenstein apples will have during the following six or eight weeks with apples from other parts of the United States.

HOW TO USE THE RESULTS OF THE PRICE ANALYSIS

The average relationship between July 1 estimates of United States apple production (A), Gravenstein apple production in the Sebastopol district (B), and the price of Gravensteins (X), for the period 1919 to 1927, is expressed in the formula

$$\overline{X} = 3.10901 - 0.00832A - 0.000839B,$$

in which A is in millions of bushels, B in thousands of boxes, and $\overline{X}$ in dollars per box. This equation indicates that for the period 1919 to 1927 each change of 1,000,000 in A, the price on an average changed 0.832 cents, and for each change of 1,000 in B the price changed 0.0839 cents—changes in price in each case being in the opposite direction from changes in quantities.

The fact that the relationship expressed by this equation has held somewhat consistently for the past nine years should make it of some value to buyers and sellers of Gravenstein apples in California in deciding on a price for apples which should move them into consumption.

AVERAGE RELATIONSHIP OF PRODUCTION AND PRICE, 1919–1927
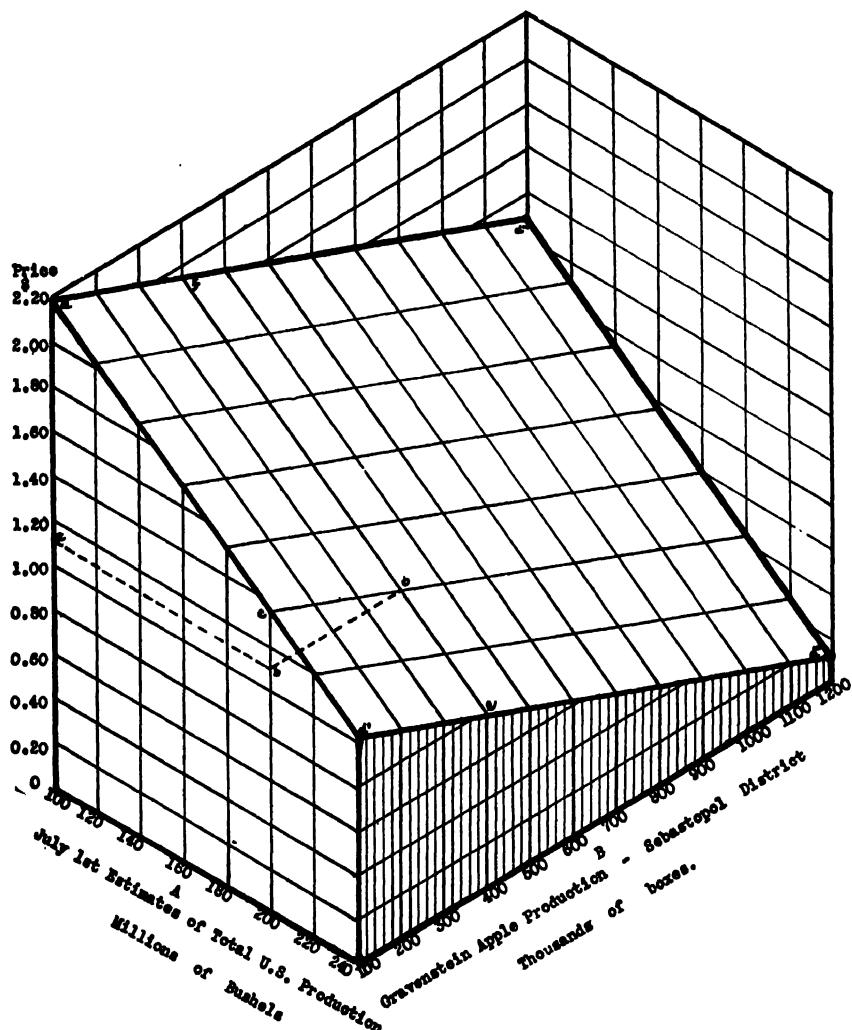
Fig. 4.—The line *dd'* and those parallel to it show the average net effect of July 1 estimates of United States production on Gravenstein apple prices with Gravenstein production held constant at various values along *B*. The line *dd'''* and those lines parallel to it show the average net effect of Gravenstein apple production in the Sebastopol district on price with United States production held constant at various values along *A*.

To illustrate the use of the equation let us assume the following conditions:

A—July 1 estimates of United States apple production = 200 million bushels.

B—Sebastopol production of Gravenstein apples = 400 thousand boxes. (Estimates of the size of the crop in the Sebastopol district can be made very accurately at the beginning of the season.)

The problem is to find $\overline{X}$—the price to the farmers which, based on past experience, will come closest to equating supply and demand.

Substituting in the equation

$$\overline{X} = 3.10901 - 0.00832A - 0.000839B$$

the above values for A and B we obtain

$$\overline{X} = 3.10901 - (0.00832 \times 200) - (0.000839 \times 400)$$
$$= 3.10901 - 1.664 - 0.3356$$
$$= \$1.11$$

The estimated price for any values of A and B within the range shown may also be obtained approximately from figure 4, which shows graphically the average effect of A and B on price from 1919 to 1927. The line dd' in figure 4 shows the average net effect on price of production estimates from 100 million to 240 million bushels with a constant production of 100 thousand boxes for B. The next line parallel to dd' shows the average net effect on price of production estimates from 100 million to 240 million bushels with a constant production of 200 thousand boxes for B. The other lines parallel to dd' including $d'''d''$ show the net effect on price of production estimates from 100 to 240 million bushels with constant productions of 300, 400, 500, etc., to 1,200 thousand boxes at $d'''d''$.

In the same way the line dd''' shows the average net effect of varying B from 100 thousand to 1,200 thousand while A is constant at 100 millon. The next line parallel to dd''' shows the same thing for various values of B with A at 120 millions. The other lines parallel to dd''' including $d'd''$ show the net effect on price of various values of B with A at values from 140 to 240 million bushels.

To illustrate the use of figure 4 in estimating the price that will equate supply and demand let us assume a value of 200 million for A and 400 thousand for B—the same values used in illustrating the use of the equation above. The line extending from 200 on the base line A to the line dd' meets it at c, which indicates a price estimate of \$1.36 with B having a value of 100 thousand. In order

to get the price estimate when B has a value of 400 thousand, follow the line from c to o parallel to $d'd''$. At o it intersects the line ef which gives the various price estimates when B equals 400 thousand and A varies from 100 million to 240 million. The point o, therefore, is the point which represents the price estimates when A equals 200 million and B 400 thousand. In order to read the price represented by o, draw a line from o parallel to B until it meets the vertical line extending from 200 to c. This intersection occurs at b. From b extend a line parallel to A which meets the price scale at a where the reading is $1.11.

TABLE 4

AVERAGE RELATION OF JULY 1 ESTIMATES OF UNITED STATES PRODUCTION,
GRAVENSTEIN PRODUCTION, AND PRICES OF GRAVENSTEINS
FROM 1919–1927

July 1 estimates of United States production millions of bushels	Gravenstein production thousands of boxes							
	100	200	300	400	500	600	700	800
	Prices of Gravensteins,* dollars							
100	2.19	2.11	2.03	1.94	1.86	1.77	1.69	1.61
120	2.03	1.94	1.86	1.78	1.69	1.61	1.52	1.44
140	1.86	1.78	1.69	1.61	1.51	1 44	1.36	1.27
160	1.69	1.61	1.53	1.44	1 36	1.27	1 19	1.11
180	1.53	1.44	1.36	1.28	1.19	1.11	1.02	0.94
200	1.36	1.28	1 19	1.11	1.03	0.94	0.86	0.77
220	1.19	1.11	1.03	0.94	0.86	0 78	0.69	0.61
240	1.03	0.94	0.86	0.78	0.69	0 61	0.52	0.44

* Based on the regression equation $\overline{X} = 3.10901 - 0.00832A - 0.000839B$.

The price estimates that will be obtained at the intersection of the various lines on the plane $d\ d'\ d''\ d'''$ of figure 4 are shown in table 4. Thus when A equals 200 and B equals 400 the price estimate $1.11 is found at the intersection of the line of price estimates having 200 on the left and the column of price estimates having 400 above. It is interesting to note that a change of 20 in A has approximately the same effect on the estimated price as a change of 200 in B. For example, values of 100 for A and 300 for B give the same estimated price of $2.03 as values of 120 for A and 100 for B. This, of course, is also apparent from the equation $\overline{X} = 3.10901 - 0.00832A$ $- 0.000839B$. For intermediate values of A and B, such as 185 for A and 740 for B, close estimates can be made from either figure 4 or table 4.

SUMMARY

The price of Gravenstein apples at Sebastopol is affected by the size of the Gravenstein crop in that district, and by the size of the total apple crop in the United States. The relative effect of the former has been increasing with the increase in the size of the crop since 1912, and the effect of the latter has been decreasing.

The extent of the competition which Sebastopol Gravenstein apples are likely to meet from the rest of the United States is indicated to some extent at the beginning of the season by the July 1 estimates of United States production which are closely correlated with July shipments of apples. From 1919 to 1927 approximately 38.5 per cent of the variations in Gravenstein prices are accounted for by variations in July 1 estimates of United States production, and 40.7 per. cent of the price variations are accounted for by variations in Gravenstein production in the Sebastopol district.

The average relationship from 1919 to 1927, between price and the important factors that have been found to affect price, is shown by the equation

$$\overline{X} = 3.10901 - 0.00832A - 0.000839B$$

in which $\overline{X}$ represents estimated price, A represents July 1 estimates of United States apple production in millions of bushels, and B represents Gravenstein apple production in the Sebastopol District in thousands of boxes. Table 4, page 337, has been prepared by substituting the various production figures in the above equation and solving for $\overline{X}$—the price, judging from past experience, that is most likely to equate supply and demand for each combination of production figures. An understaning of the relationships that have prevailed in the past should be of value to any organization of Gravenstein apple growers in the Sebastopol district that wishes to have some basis for estimating at the beginning of the season, the price which is likely to bring about this equilibrium between supply and demand.

ACKNOWLEDGMENTS

SOME HOST PLANTS OF CURLY TOP

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(Contribution from the Division of Entomology and Parasitology, College of Agriculture,
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Agriculture, Bureaus of Entomology and Plant Industry.)

INTRODUCTION

Sugar-beet curly top, transmitted by the beet leafhopper, *Euteltix tenellus* (Baker), affects many species of plants among both cultivated plants and weeds. In years when a disastrous outbreak of curly top occurs among sugar beets in the western part of the United States, other cultivated plants are seriously damaged by the disease.

Forty thousand acres of beets were planted in the San Joaquin Valley during the season of 1918–19. Thirty thousand acres affected with curly top were plowed under or were not worth harvesting, and the beet leafhoppers were thus forced to seek other food plants.

During 1919, cantaloupes were a failure in the San Joaquin Valley. There was no evidence of a root rot, although root knots caused by the garden nematode, *Heterodera radicola,* were found on some of the plants examined. The trouble was attributed to a cold spring followed by warm weather, to the use of cold irrigation water, and later to a shortage of water. During the past two years cantaloupes were found to be naturally infected with curly top in the Salinas Valley and the symptoms resembled those observed in the San Joaquin Valley during 1919.

Spinach was found to be naturally infected with curly top in the San Joaquin Valley in 1919, and in many other localities in later years.

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[2] Research Assistant in Experiment Station, Agent in Bureau of Entomology, United States Department of Agriculture.

During 1924, Carsner[3, 4] came to the conclusion, on circumstantial evidence, that beans were naturally infected with curly top in Idaho. The experiments reported in this paper have demonstrated that a large number of field and garden beans growing in California are naturally infected with and susceptible to the disease.

During 1925 and 1926, curly top was transmitted to sugar beets from many varieties of squashes and pumpkins naturally infected in the field in California. The transfer of curly top from squashes and pumpkins which had been experimentally inoculated with curly top by infected beet leafhoppers, and which showed typical symptoms, back to sugar beets was also accomplished.

McKay and Dykstra[5] made a comparison of the symptoms of squash infected with the beet leafhopper with symptoms observed in the field and "circumstantial evidence suggested that the disease was due to the virus of sugar-beet curly top." The general failûre of squash in the Northwest during 1926 was due largely to this disease.

An investigation was undertaken to determine what economic plants and weeds are naturally infected with the disease. Experiments were also conducted in the greenhouse to ascertain what varieties of cultivated plants are immune, resistant, and susceptible to curly top. A list of cultivated plants immune to the disease will appear in a future paper. A study of the symptoms of the disease in varieties experimentally infected was made so that naturally infected plants could be recognized in the field. The longevity of the adult leafhopper was determined and also the cultivated plants on which their life history could be completed. Observations were made on the relation of the infection of cultivated plants to the spring dispersal, migrations, autumn dispersal, and flights of the insects occurring with the plowing under of badly diseased sugar beets. This paper gives the results of curly-top investigations concerning economic plants and weeds of the families Chenopodiaceae, Leguminosae, and Cucurbitaceae. The work has been extended to other families and the data will appear in future publications.

METHODS OF TESTING PLANTS NATURALLY INFECTED WITH CURLY TOP

Several methods were used in determining whether economic plants and weeds were naturally infected with curly top. In the early work[6, 7] began in 1918, cultivated plants and weeds on which the beet leafhoppers were found were removed from the field and enclosed in cages in the greenhouse. Nymphs which hatched from

eggs deposited in the plants under natural conditions were fed in cages on the plants. As these plants usually became dry in the cages in from one to two weeks, several potted sugar beets were put into the cages to allow the nymphs to complete their life cycle. If the plants were infected with curly top under natural conditions, the nymphs transmitted the disease from the plants to the beets. This method had a twofold function: to determine, first the natural host plants of the beet leafhopper; and second, the cultivated plants and weeds which were naturally infected with curly top.

A simple method was adopted later in testing naturally infected plants. Male beet leafhoppers non-infective as to curly top were fed on stunted diseased plants removed from the field, and then transferred to healthy beet seedlings. Males were used rather than females so as to avoid egg deposition. If the beets developed curly top it was evident that the cultivated plants and weeds had been naturally infected with the disease. In each case the number of non-infective males which fed on a plant suspected of harboring curly top varied from ten to twenty-five or more. A high mortality of the insects often occurred in the greenhouse owing to unfavorable food. The hoppers were transferred, according to their death rate, to two or more beets. For instance, when twenty-five males were used and some died, the remainder were equally divided between two cages, each enclosing a beet. If the symptoms of curly top failed to develop in from one to two weeks the beets were examined daily in insect-proof chambers for a period of six weeks. Checks were often used in which apparently healthy cultivated plants or weeds were removed from the field, and cross inoculations were made with non-infective leafhoppers feeding on the plants, to beet seedlings.

METHODS OF EXPERIMENTALLY INFECTING PLANTS WITH CURLY TOP

A large number of cultivated plants and weeds were experimentally infected with curly top so that the symptoms of naturally infected plants could be recognized in the field. The plants were grown from seeds in a greenhouse which was fumigated twice a month with nicotine sulphate. From two to ten infective male beet leafhoppers confined in cages were used to inoculate the plants, the number depending upon the size of the plant. When the longevity of the adults was short because of unfavorable food, the plants were repeatedly inoculated with different lots of males. The period allowed

for inoculation was usually two weeks, or less, if symptoms of curly top developed earlier. After the period of inoculation, the cage containing the infective males was removed from the plant. In another cage non-infective males were fed on the inoculated plant, for a period of at least two or three days, or longer if the food material was favorable. The leafhoppers were then transferred from the inoculated plant to healthy beet seedlings. If the beet developed curly top, it was evident that the inoculated plant had been infected with the disease.

FLIGHTS OF BEET LEAFHOPPER

Spring Dispersal.—After the pasture vegetation becomes dry on the plains and foothills of a natural breeding ground, the spring-brood females fly into the adjacent cultivated areas. Most of the males remain behind on the plains and foothills and die. The invasion is not a single flight. The insects invade the cultivated regions in the San Joaquin Valley during a period of from four to ten weeks.

Spring Migration.—The appearance of the beet leafhoppers in the Sacramento Valley seems to be associated with a spring migration, probaby a northward movement from the San Joaquin Valley. The evidence for a spring migration hinges on the fact that from 1918 to 1928 the insects did not invade the cultivated areas until some time after the pasture vegetation became dry on the foothills of the Coast Range.

Flights Associated with Unfavorable Food.—Flights of the beet leafhoppers occur in the cultivated areas when the food material becomes unfavorable. When the outer leaves of badly diseased beets become sun-scorched during hot weather, and the remaining tuft of diseased leaves become thick and leathery, many of the summer-brood adults seek other food and breeding plants. The insects will also desert badly diseased weeds. The hoppers will leave large beets with dense foliage covering the rows, and fly to more favorable host plants, often to smaller beets in the vicinity. When certain species of annual saltbushes become woody in July, a dissemination of the leafhoppers to other plants occurs. In the struggle for food of the ever-increasing hordes of bugs during the summer, random flights occur, and in all probability some of the insects fly into fields of other crops and transmit curly top.

Autumn Dispersal.—During October and November the over-wintering adults fly from the cultivated areas of the San Joaquin Valley to the plains and foothills. In the Salinas Valley the leaf-

hoppers fly to the foothills, following the Salinas River and its tributaries.

Plains and Foothill Breeding Areas.—The foothill breeding area of the beet leafhopper in the San Joaquin Valley extends from Mt. Diablo to the Tehachapi Mountains on the Coast Range, also the foothills of the Tehachapi and Sierra Nevada Mountains as far north as Round Valley near Lindsay. The natural breeding grounds also include the plains of the middle San Joaquin Valley and most of Kern County in the southern section of the valley.

During years with early autumn rains, the beet leafhoppers were taken on the foothills of the Coast Range bounding the Sacramento Valley, but during the winter the hoppers were exterminated. No hold-over bugs were taken in the early-planted beet fields nor was a single case of curly top observed until after the migratory flights occurred. In all probability, the factors which exterminate the over-wintering adults in the Sacramento Valley are heavy fogs and rain-fall. The normal rainfall in this valley varies from 19.28 to 27.75 inches. The hot dry summers in the Sacramento Valley are favorable to the immigrants and later generations in the cultivated areas.

CHENOPODIACEAE, GOOSEFOOT OR SALTBUSH FAMILY

CURLY-TOP SYMPTOMS ON THE SUGAR BEET (BETA VULGARIS)

Reliable and constant symptoms of sugar-beet curly top are not always present in all cultivated plants and weeds and hence all visible symptoms of beet curly top will be described as a basis for comparison with the characteristics of the disease in other plants.

Leaf Curling.—The earliest symptoms of curly top to appear in most diseased beets is an inward rolling of the lower and outer margin of the youngest leaves. Later the entire blade may show a pronounced inward curling toward the mid-rib (figs. 1 and 2). There is a considerable variation as to the number of curled leaves occurring in older diseased beets, but very often the outer full-grown leaves do not show this character. A beet showing curly leaves and no other symptoms of the disease is not always a curly-top beet, for perfectly healthy beets may show the same characteristic.

The foliage of some diseased beets shows an outward rolling of the margin of the leaves and an outward puckering of the blade between the veins. Sometimes the two types of leaf curl are combined, the blade curling outward and the margin inward.

Fig. 1. Side view of sugar beet (*Beta vulgaris*) affected with curly top, showing inward curling of leaves toward the mid-rib.

Fig. 2. Top view of same beet as that shown in figure 1, showing inward curl of leaves.

Blister-like Elevations.—A symptom of curly top which sometimes develops on the leaves of beet seedlings is small blister-like elevations (pl. 1, figs. 1, 2). In beet seedlings with four to six leaves including the cotyledons, these blisters may appear simultaneously on the outer and the inner leaves, or on an outer leaf before the youngest leaf is developed, or on only the youngest leaf. The blister-like elevations sometimes develop in two days after the beet seedling is infected with the disease.

Transparent Venation.—A reliable and constant symptom of curly top plainly visible to the eye is the transparent network of minute veins (pl. 2, fig. 2), generally occurring on the innermost or youngest leaves of the beet. At the beginning this symptom may be confined to a portion of the youngest leaf, but in a few days, in vigorously grow- ing beets, the entire leaf is affected. Sometimes the cleared veinlets and blister-like elevations appear simultaneously on the youngest leaf of beet seedlings (pl. 1, fig. 3). The transparent veinlets sometimes appear on the youngest leaf of beet seedlings within two days after infection with the leafhopper. In older beets in the field, this symptom may develop in from one to two weeks or longer after infection   A diseased beet may retain the transparent venation on the leaves during the entire season and show no other symptom. Late-infected beets suffering from lack of moisture may show the cleared veinlets on the youngest leaves and no other symptom.

Protuberances on Leaves.—Another reliable and constant symptom of curly top is the roughened appearance of the lower surface of the leaves, developing usually after the veinlets have become transparent. A closer examination of this roughened condition upon its first appearance reveals numerous small elevations on the veins resembling tiny warts (pl. 3, figs. 1, 2). As the disease progresses, nipple-like papillae and knot-like swellings (pl. 3, fig. 3) resembling galls develop here and there on the distorted veins. The diseased leaves are dark, dull green in color, thick, crisp, and brittle.

Exudation from Leaves.—When a large number of curly-top beets are examined in the field, an occasional plant may show a few drops of clear viscid liquid exuding from the petioles, mid-rib, or veins on the lower surface of the leaves. Later this liquid becomes black (pl. 3, fig. 4) and sticky, and upon drying forms a brown crust. This syrupy substance often oozes out of many diseased beets after the first irrigation, and attracts enormous numbers of insects which feed upon the sweet drops of beet juice.

Yellowing.—When curly-top beets are irrigated, they sometimes show a temporary improvement, but later the leaves often turn yellow. It is not to be inferred, however, that the yellowing of the foliage occurs only after the fields have been irrigated; the leaves of diseased beets, especially young plants, will turn yellow without irrigation.

Fig. 3. Cross and longitudinal sections of beets affected with curly top. The transverse sections show black concentric rings alternating with light areas. The longitudinal sections show the dark discolorations extending lengthwise through the beet.

Hairy Roots.—When a badly diseased beet is pulled from loose soil, particles of dirt sometimes cling to the rootlets and shake off with difficulty. It is evident that there is an increase in the number of rootlets, a condition which has been described as "hairy root" or "woolly root" or "whiskered beets." In harder soil these roots often tear off when the beet is pulled.

Darkened Rings in Beet Root.—A cross section of a diseased beet often shows black concentric rings which alternate with light areas (fig. 3). A longitudinal section shows the dark discoloration extending lengthwise through the beet.

BETA MARITIMA

Brandes and Klaphaak[1] have introduced seeds from *Beta maritima* and other wild species of *Beta* and propose to cross the primitive with the cultivated beets to ascertain if strains resistant to curly top and other diseases can be developed. *B. maritima* was naturally infected with curly top at Spreckels, California, during the 1925 outbreak of the beet leafhopper. The sugar beet (*Beta vulgaris*) is presumably a derivation of *B. maritima* indigenous to the Mediterranean regions of Europe.

MANGEL WURZEL OR STOCK BEET (BETA VULGARIS)

Mangel wurzel or stock beets planted during April at Berkeley were found to be naturally infected during the serious outbreak of the beet leafhopper in 1925. The same varieties planted on the University Farm at Davis were so badly affected with curly top that it was impossible to make a comparative yield test of the two plantings.

The following varieties were naturally infected with curly top: Giant Yellow, Golden Tankard, Half Sugar, Mammoth Long Red, Red Eckendorf, Yellow Eckendorf, and Sludstrup. The reliable foliage symptoms of curly top on mangel wurzel or stock beets are similar to those on the sugar beet.

Nymphs in all stages of development were found on the naturally infected varieties of mangel wurzel or stock beets, and in all probability, the life history was completed on these food plants.

GARDEN, TABLE, OR RED BEET (BETA VULGARIS)

All varieties of garden beets grown in California are naturally infected with curly top. During a severe outbreak of curly top, late-planted garden beets grown in the interior regions of the state are often badly stunted. The foliage symptoms of curly top on garden beets are similar to those on the sugar beet.

SWISS CHARD (BETA VULGARIS CICLA)

Swiss chard has been found to be naturally infected with curly top in many localities of California. This plant was badly diseased in the vegetable garden of the Spreckels ranch near King City during 1926 when no general outbreak of the beet leafhopper occurred in the state.

The varieties experimentally infected are Giant Lucullus, Improved Silver, and Large Ribbed White. The reliable foliage symptoms of curly top in Swiss chard (fig. 4) are similar to those in the sugar beet.
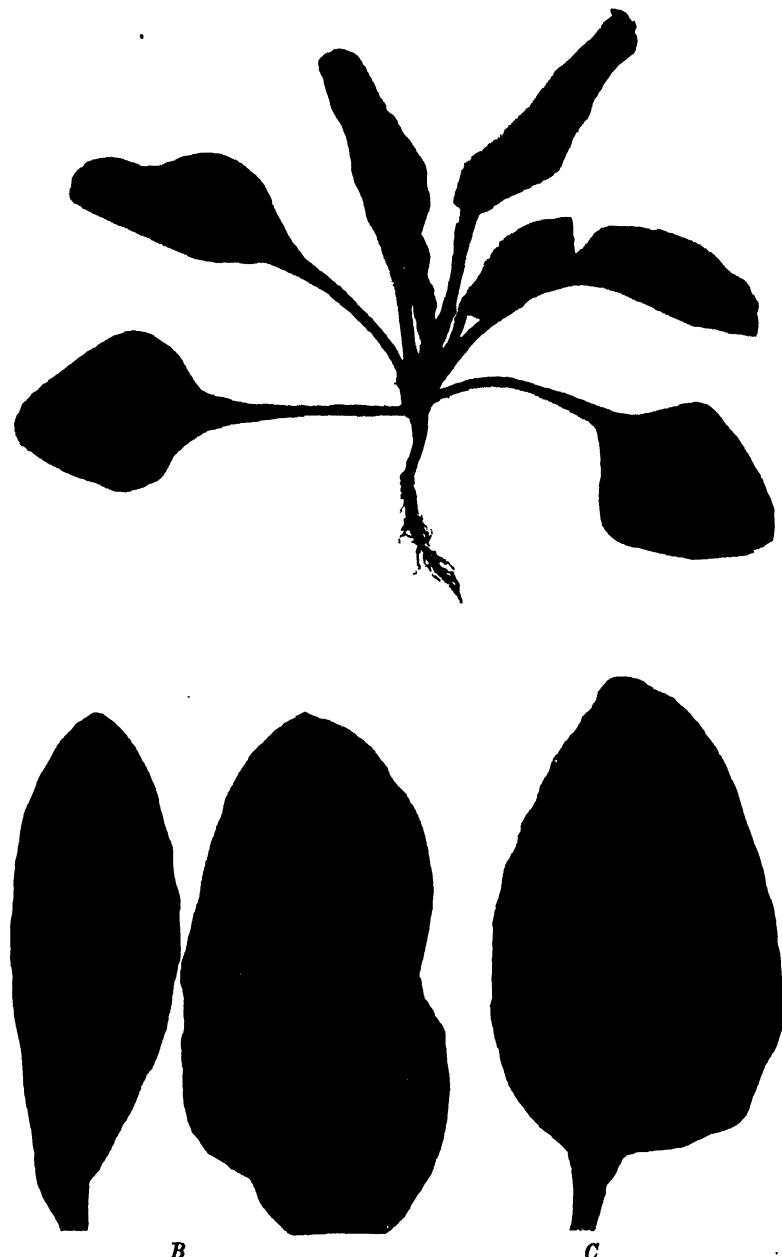
Fig. 4. Swiss chard (*Beta vulgaris cicla*): *A*, plant naturally infected with curly top, showing protuberances on inward-curled leaves (King City, Salinas Valley, September 7, 1926). *B*, left, transparent venation; right, normal venation of healthy leaf. *C*, protuberances on lower surface of leaf affected with curly top.

SPINACH (Spinacia oleracea)   ·

During the 1919 outbreak of the beet leafhopper, spinach grown in the San Joaquin Valley was found to be naturally infected with curly top.   In 1925 it was demonstrated that spinach was infected

Fig. 5.   Prickly Seeded spinach (*Spinacia oleracea*): *A*, left, healthy plant used as a check or control on which non-infective beet leafhoppers fed; center and right, plants infected with curly top showing inward-curled leaves. *B*, right, portion of healthy check or control plant; portions of three other plants show-ing rolled, inward-curled and outward-cupped leaves respectively.

with the disease in the Sacramento Valley.   One crop of spinach grown on the University Farm at Davis turned yellow and died.   An examination of another planting of Bloomsdale Savoy spinach showed that the beet leafhopper was present but scarce on September 23.

Nine spinach plants in different stages of disease were selected, the leaves of two of which were yellow. Non-infected leafhoppers transmitted curly top from seven plants, including the two yellow plants, to beet seedlings. The bugs failed to transmit the disease from two diseased spinach plants to beets, indicating that the spinach may have suffered also from some other trouble. During 1926 spinach was found to be naturally infected with curly top in the Santa Clara Valley.

The following varieties were experimentally infected with curly top: Bloomsdale Savoy, Long Standing, Round Summer, Prickly Seeded, New Zealand, and Virginia Savoy. The youngest leaves of these varieties showed a clearing or transparency of the minute veins,

Fig. 6. Virginia Savoy spinach (*Spinacia oleracea*) showing youngest leaves rolled toward petioles.

but this symptom is often difficult to distinguish from the normal venation. The leaves may develop an inward curl (fig. 5*A*) or roll (fig. 5*B*), as in Prickly Seeded spinach, or an outward curl or roll toward the petiole (fig. 6), as in Virginia Savoy spinach. Later the young plants turned yellow and died as they do in the field.

LIFE HISTORY OF BEET LEAFHOPPER ON CULTIVATED CHENOPODS

Nymphs which hatched from eggs deposited in the following varieties of cultivated plants of the Chenopodiaceae completed their life cycle on these host plants in the greenhouse: sugar beet; *Beta maritima;* garden, table, or red beets; Giant Lucullus, Improved

Silver, and Large Ribbed White Swiss chard; Bloomsdale Savoy, Long Standing, Round Summer, Prickly Seeded, New Zealand, and Virginia Savoy spinach.

WEEDS AND SHRUBS OF THE CHENOPODIACEAE

The following weeds were found to be naturally infected with curly top: bractscale (*Atriplex bracteosa*); redscale, or red orache (*A. rosea*); silverscale, or fog weed (*A. argentea expansa*); and spearscale, or spear orache (*A. patula hastata*); *Chenopodium leptophyllum*; nettle-leaf goosefoot (*C. murale*); Mexican tea (*C. ambrosioides*); and Russian thistle (*Salsola kali tenuifolia*).

The following weeds and shrubs were experimentally infected with curly top: arrowscale (*Atriplex phyllostegia*); *A. tularensis*; bractscale (*A. bracteosa*); brittlescale (*A. parishi*); crownscale (*A. coronata*); heartscale (*A. cordulata*); redscale, or red orache (*A. rosea*); silverscale, or fog weed (*A. argentea expansa*); and spearscale (*A. patula hastata*); Australian saltbush or fleshscale (*A. semibaccata*); ballscale (*A. fruticulosa*); and quail brush or lenscale (*A. lentiformis*); *Chenopodium leptophyllum;* lamb's quarters (*C. album*,); nettle-leaf goosefoot (*C. murale*); Mexican tea (*C. ambrosioides*); and soap plant (*C. californicum*); *Nitrophilo occidentalis*; and Russian thistle (*Salsalo kali tenuifolia*).

The following perennial saltbushes were found to be non-susceptible to curly top: cattle spinach or allscale (*Atriplex polycarpa*) and spinescale (*A. spinifera*).

Recovery from Disease.—*Chenopodium leptophyllum* growing in beet fields in the San Joaquin Valley was occasionally observed with an apparently healthy branch growing from a stunted plant with yellow curled leaves (fig. 7*B*), a phenomenon which may be associated with recovery from the disease.

Longevity of Virus in Perennials.—The longevity of the virus was determined in Mexican tea (*Chenopodium ambrosioides*), a perennial, which was tested and shown to be naturally infected during 1925. Four plants were kept in insect-proof chambers in the greenhouse for one year and during 1926 non-infective beet leafhoppers repeatedly transmitted curly top from the four plants to beet seedlings. On the other hand, quail brush or lenscale (*Atriplex lentiformis*) was experimentally infected with curly top, but one year later non-infective beet leafhoppers failed to transmit the disease from any of the eight plants tested. The eight plants were then reinfected and curly top was again transmitted from two of the quail brush to sugar beets.

Resistant Weeds.—Weeds often show a high degree of resistance to curly top, in fact, some individuals of a species are immune to the disease. Thirty Australian saltbushes (*Atriplex semibaccata*) grown from seeds were repeatedly inoculated with different lots of infective beet leafhoppers, but it was impossible to infect twenty-seven plants.

A B

Fig. 7. *Chenopodium leptophyllum: A,* healthy field check or control plant from which non-infective beet leafhoppers failed to transmit curly top to sugar beets. *B,* plant naturally infected with curly top, showing curled leaves on one branch and normal leaves on an apparently healthy stem (Manteca beet field, San Joaquin Valley, July 21, 1925).

The details concerning the methods used with the three infected plants are worthy of mention. Non-infective males transmitted curly top from the first infected plant to one beet seedling, but another beet failed to develop the disease. One lot of non-infective males communicated curly top from the second infected plant to a beet, but another lot of non-infective males allowed to feed on the Australian

saltbush twenty-three days later failed to transmit the disease. The disease, however, was transmitted from the third infected plant by two lots of non-infective males in tests conducted exactly as in the case of the second Australian saltbush.

Cattle spinach or allscale (*Atriplex polycarpa*) and spinescale (*A. spinifera*) are listed as non-susceptible to curly top, but more tests are necessary before it is definitely established that these plants are immune from the disease.

Natural Breeding Plants of Beet Leafhopper.—The beet leaf-hopper has been bred from eggs deposited in all the weeds listed, except *Atriplex tularensis*, quail brush or lenscale (*A. lentiformis*), cattle spinach or allscale (*A. polycarpa*) and soap plant (*Chenopodium californicum*). The plants upon which leafhoppers were collected were removed with the root system from the field and the nymphs which hatched were reared to the adult stage. It is evident that the eggs were deposited in the weeds under natural conditions and by this method the females were not forced to oviposit in the plants.

One condition greatly favoring an increase of the beet leafhopper in the San Joaquin Valley is the abundance of the breeding plants in the cultivated areas. The plants upon which enormous numbers of nymphs and adults are taken in the field are representatives of the goosefoot or saltbush family (Chenopodiaceae). After the flights of the adults from the plains and foothills into the cultivated regions cease in the San Joaquin Valley during a severe outbreak of the pest, the insects are far more abundant on weeds of the Chenopodiaceae and closely related families than on sugar beets. In the Salinas Valley the most favorable weeds are not so abundant and the multiplication of the hoppers occurs chiefly on the beets.

LEGUMINOSAE, PEA FAMILY

BEANS (PHASEOLUS VULGARIS AND P. LUNATUS)

Destructiveness of Curly Top to Beans in Idaho.—During the serious outbreak of the beet leafhopper in Idaho in 1924, a disastrous epidemic disease of beans occurred in Twin Falls County. Carsner[3, 4] came to the conclusion that the beet leafhopper may have transmitted curly top to beans, although he did not see the disease in the field. He demonstrated, however, that seven varieties of beans commonly grown in Twin Falls County were susceptible to curly top, using the method previously described. The fact that the seven varieties were sus-

ceptible to curly top by inoculating the plants with infective leaf-hoppers does not prove that any of the varieties were infected with the disease in the field.

Injury in California.—A survey of curly-top infection of field beans was made in the Salinas, San Joaquin, and Sacramento valleys of California during the severe outbreak of the disease in 1925. In the interior regions of the Salinas Valley, Small White beans (*Phaseolus vulgaris*) were seriosly affected with the disease. In a bean field consisting of 481.5 acres on Ranch No. 3 of the Spreckels Sugar Company, near King City, the percentage of curly top was determined in two adjacent fields containing 30.4 and 46.9 acres respectively. The details concerning the dates of planting and plowing under of sugar beets, dates of planting Small White beans, and the percentage of curly top follows.

FIELD A	FIELD B
(30.4 acres)	(49.6 acres)
Feb. 26, beets planted.	Feb. 5, beets planted.
June 11, beets replanted.	June 6, beets plowed under.
July 1, beets plowed under.	June 8, beans planted.
July 3, beans planted.	August 20, beans, 63 per cent curly top.
August 20, beans, 15 per cent curly top.	

The average yield of the 481.5 acres was 4.84 sacks to the acre, but no separate record was taken of fields A and B. Field B was not planted early enough to mature in time to make a full crop. The average yield of field beans, except limas, in California from 1920 to 1924 was 7.7 sacks to the acre. The reduction in the average yield of 2.86 sacks to the acre can to a large extent be attributed to curly top, although minor bean troubles may also have been a factor.

Relation of Spring Flights to Curly-Top Appearance in Bean Fields.—Small White beans were not infected with curly top during the large spring flights which occurred on March 24 to 26, and April 12 to 14, in the Salinas Valley, since the beans were not planted in the two fields until June 8 and July 3. A partial second brood developed on the foothills of the Salinas Valley and the hoppers were still present on the hills on June 5. Some of the second brood adults may have invaded the bean fields.

Relation of Spring Fights to Dates of Planting Beans.—Beans were planted from April 25 to May 1, 1925, on the Spreckels ranches in the Salinas Valley. It is evident that these plantings also escaped the large spring flights of the beet leafhopper.

Flights.—Associated with Plowing Under of Sugar Beets.—It appears probable that after the beets were plowed under on July 1, in field A, the beet leafhoppers flew into the bean fields planted on June 8, in the adjacent field B. From January 4 to February 26, 3,000 acres of beets were planted on this ranch; 2,330 acres were plowed under after curly top developed; 192 acres were replanted from June 6 to 13, and again plowed under in July, forcing the bugs to seek other food plants.

Flights Associated with Harvesting Sugar Beets in the Sacramento Valley.—During the harvesting of the sugar beets in the Sacramento Valley, a number of bean fields were examined in the vicinity of beet fields, and enormous numbers of leafhoppers were found on different varieties of beans. The beans were nearing the ripening period, and curly top probably did not seriously reduce the yield. During 1926 a field of Henderson Bush Limas was swarming with the pest after the beets in an adjacent field had been harvested. A number of plants were tested by the simple method previously described and found to be naturally infected with curly top.

Small Whites, an Unfavorable Host Plant of Beet Leafhoppers.—Small Whites removed from the field near the ripening period proved to be unfavorable food and breeding plants for the beet leafhoppers. The longevity of the adults varied from seven to seventeen days in captivity on this variety of bean, but under natural conditions, they could feed on favorable weeds growing in bean fields. By shaking many Small White bean plants in the two fields a nymph would hop to the ground on very rare occasions, but these may have crawled on the plants from weeds. Nymphs which hatched from eggs deposited in Small Whites failed to acquire the winged stage.

Varieties Naturally Infected.—An examination of other varieties of beans grown in the interior regions of the Salinas Valley such as Bountiful, Cranberry, Kentucky Wonder, and White-Seeded Kentucky Wonder, all classified as *Phaseolus vulgaris*, showed that from 1 to 5 per cent were infected with curly top. The percentage of curly top of Henderson Bush Lima (*P. lunatus sierra*) could not be determined, as no typical foliage symptoms could be detected. Stunted plants with the younger leaves dwarfed and puckered were tested and found to be naturally infected with the disease. These varieites of beans were not replanted from beets to beans, nor were any of these bean fields in the immediate vicinity of beet fields that had been plowed under. The variety Cranberry was found to be infected with curly top in the fog belt at Spreckels.

In the Sacramento Valley the Long Red Kidney bean growing in the vicinity of beet fields that had been harvested was found to be naturally infected with curly top. Foliage symptoms resembling bean curly top were apparently caused by a stem and root rot. A variety of bean known as Stringless Green Pod grown at the University Farm at Davis was also found to be infected with curly top.

Stunted pink beans have not been found to be naturally infected with curly top up to the present time in the Salinas, San Joaquin, and Sacramento valleys. Pink beans in the vicinity of beet fields which had recently been harvested were swarming with the beet leaf-hopper but non-infective males failed to transmit curly top from this variety of bean to beets.

Field Checks.—Cross inoculations with non-infective males feeding on different varieties of apparently healthy field and garden beans removed from the field as a check failed to transmit curly top to sugar beets.

Experimentally Infected Varieties.—The following varieties of field beans grown in California were experimentally infected with curly top: Bayo, Blue Pod, Cranberry, Lady Washington, Pink, Red Kidney, Red Mexican, Small White, and Spotted Red Mexican, all classified as *P. vulgaris;* also the following varieties of limas (*P. lunatus*): Burpee's Bush, Fordhook Bush, Lewis, and Baby Lima or Henderson Bush. The following varieties of garden beans were tested and found to be susceptible to curly top: Bountiful, Early Refugee, Golden Wax, Kentucky Wonder Pole, Kentucky Wonder Wax, Prolific Black Wax, Scarlet Runner Pole, Stringless Green Pod, White Crease-back, and White-Seeded Kentucky Wonder.

Symptoms.—A study was made of the development of the symptoms of curly top in the common California varieties of field and garden beans grown from seeds. After the cotyledons pushed through the soil and the first pair of leaves appeared, each variety was inoculated by ten infected beet leafhoppers confined in a cage. If the longevity of the adults was short, repeated lots of ten hoppers were put into the cage until symptoms of curly top appeared. The first symptoms to appear in from one to two weeks with varieties of *Phaseolus vulgaris* were a puckering and an outward cupping of the newly developing leaves, with a clearing or transparency of the minute veins. The youngest leaves were decidedly dwarfed and darker green.

In naturally infected Small Whites the cupping of the three leaflets sometimes continued until each leaf resembled a small green

A

B

Fig. 8. Small White bean (*Phaseolus vulgaris*): A, stunted plant naturally infected with curly top, showing compact dense mass of balled inner leaves. B, the same plant with the outer leaves removed and the central mass teased apart, showing the balled leaves formed by an outward cupping of the three leaflets (King City, Salinas Valley, August 19, 1925).

ball (fig. 8*A*). Figure 8*B* shows a plant with the outer leaves removed and the central mass of balled inner leaves teased apart.

The varieties of Limas infected in the greenhouse do not show pronounced foliage symptoms of curly top except dwarfing, puckering, and slight cupping of the youngest leaves. Transparent venation,

Fig. 9. Small White bean (*Phaseolus vulgaris*): three bean pods on the left from a healthy plant; eight dwarfed pods on the right, the only pods found on eight plants naturally infected with curly top (King City, Salinas Valley, October 9, 1925).

a reliable symptom of curly top, was usually absent. An occasional infected Henderson Bush Lima showed faint indications of cleared veinlets on the first pair of true leaves.

An early-infected Small White bean plant usually bears no pods. Eight Small Whites naturally infected with curly top were pulled at random in the field on October 8, and there were only eight dwarfed pods on all of the plants (fig. 9).

Fig. 10. Pink bean (*Phaseolus vulgaris*): *A*, plant showing puckering and cupping of youngest leaves, resembling curly-top symptoms. This plant was repeatedly inoculated with different lots of infective beet leafhoppers but non-infective males failed to transmit curly top to sugar beets after feeding on the inoculated plant. *B*, Pink bean infected with curly top by infective beet leafhoppers, showing puckered and cupped leaves. Non-infective males repeatedly transmitted curly top to sugar beets from this plant during a period of three months. *C*, puckered and cupped leaves of Pink bean infected with curly top. *D*, *E*, puckered and cupped leaves of Pink bean from which the disease was not transmitted.

Races of the Pink Bean Resistant to Curly Top.—Carsner[2] states that Pink beans are non-susceptible to curly top.

Pink beans were repeatedly inoculated with different lots of ten infective adults but only three of thirty-seven plants were susceptible to the disease. Typical symptoms of bean curly top (fig. 10*B*, *C*) developed in the three infected plants; some of the other plants inoculated by infective hoppers showed similar symptoms (fig. 10*A*,

D, E) yet non-infective males failed to transmit the disease from the latter to sugar beets. The youngest leaves of many inoculated plants would show a slight puckering and outward cupping, but a few days later these leaves would be normal. It was assumed that possibly the virus remained active for a short time. Experiments were so conducted that non-infective insects could feed on only the youngest puckered leaves for a period of from two to seven hours or longer under high temperatures, and yet the hoppers failed to transmit the disease to healthy beet seedlings. A later experiment with one of three Pink beans infected with curly top during July, demonstrated

TABLE 1

LONGEVITY OF LAST LIVING MALE AND FEMALE OF TWENTY BEET LEAFHOPPERS
ON FIELD BEANS

Variety of Bean	Lon-gevity males *days*	Temperatures			Lon-gevity females *days*	Temperatures		
		Max. °F	Min. °F	Mean °F		Max. °F	Min. °F	Mean °F
Bayo	2-3	102	58	78.9	18	102	58	80.0
Blue Pod	3-4	102	58	79.1	7	102	58	79.2
Cranberry	4	102	58	79 3	12	102	58	79.5
Lady Washington	2-3	102	58	78.9	8	102	58	79.1
Pink	2-5	102	58	79.5	12	102	58	79.5
Red Kidney	2	102	58	78.9	9	102	58	79.3
Red Mexican	3	102	58	79.2	10	104	58	80.1
Small White	4	102	60	79.4	11	102	58	79.4
Spotted Red Mexican	2-4	104	60	81.6	10	104	60	81.6
Henderson Bush Lima	4-5	104	60	80.9	7-14	104	60	79.8
Lewis Lima	2-4	104	60	81 0	9	104	60	81.0

that the virus remained active during August and September, since the disease was repeatedly transmitted to beet seedlings. In all probability, races susceptible to curly top occur in the mixture of races in Pink beans, while some strains are highly resistant or immune.

Longevity of Beet Leafhoppers.—The longevity of male and female beet leafhoppers feeding on field beans under high temperatures of the greenhouse is indicated in table 1. Spring or summer-brood adults were used and not the dark overwintering forms. Ten leafhoppers of each sex were fed on two or more bean plants of each variety and the adult life of the last living male and female is recorded in table 1.

It is evident from table 1 that the females live longer than the males on a bean diet.

Curly-top Transmission from One Plant to Another of the Same Variety.—Non-infective beet leafhoppers transmitted curly top from Small Whites naturally infected in the field to three Small Whites

grown from seeds. Non-infective males also communicated the disease from the three infected Small Whites to beets. Similar results were obtained with naturally infected Henderson Bush Limas.

Curly-Top Transmission from One Variety to Other Varieties.—
An experiment similar to the preceding was conducted except that the leafhoppers were transferred from a naturally infected Small White to Kentucky Wonder, Henderson Bush Lima, and Early Refugee beans grown from seeds. All varieties of beans developed curly-top symptoms. Non-infective hoppers also transmitted the disease from the three varieties of beans to beets.

The transmission of curly top from one variety to other plants of the same variety or of different varieties of beans is rarely accomplished by male beet leafhoppers under greenhouse conditions owing to the short life of the males on a bean diet. This may not be the case under natural conditions as the leafhoppers are not limited to a bean diet but can feed on favorable weeds.

COWPEA (Vigna sinensis)

Several fields of Blackeye cowpeas were located in the San Joaquin Valley with no beet fields in the vicinity. An occasional beet leafhopper was captured by sweeping the Blackeyes with an insect net. Stunted plants with yellow leaves were tested and found to be naturally infected with curly top.

The varieties of cowpeas susceptible to experimental inoculation with the disease were Blackeye and Whippoorwill or Speckled. Blackeyes were repeatedly inoculated by different lots of ten infective beet leafhoppers but only four of the ten plants tested were proved to be susceptible to the disease. The two varieties of cowpeas showed no reliable foliage symptoms of curly-top; the infected plants, however, were stunted with the leaves slightly yellow. The longevity of the leafhoppers on the two varieties of cowpeas was as follows

	Males	Females
Blackeye	1–3 days	5–13 days
Whippoorwill	2–5 days	8–12 days

HORSE BEAN (Vicia faba)

The following varieties of Horse beans were tested and found to be susceptible to curly top: Broad Windsor or Horse bean; Small Windsor or New Zealand Horse bean, and Bell Windsor or Small-seeded Horse bean.

Fig. 11. Youngest leaves of Horse bean (*Vicia faba*), showing inward-curled leaves and blister-like elevations, symptoms of curly top.
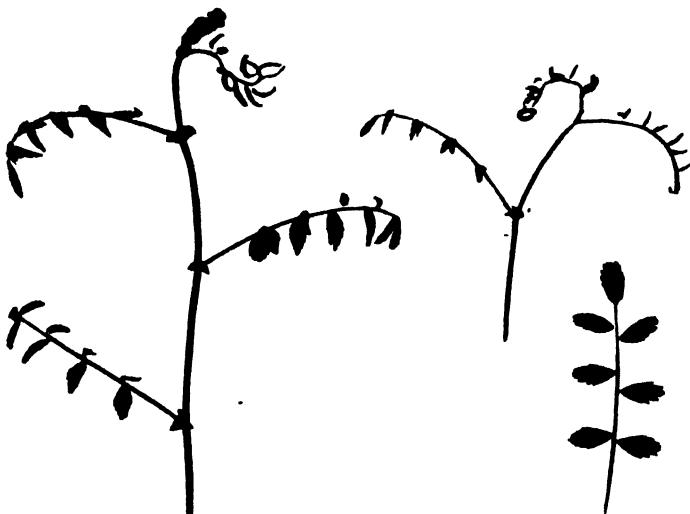
Fig. 12. Garbanzo or Chick-pea (*Cicer arietinum*) infected with curly-top, showing distorted mid-rib of compound leaves and inward-curled leaflets.

The three varieties of Horse beans infected with curly top in the greenhouse showed an inward curl, blister-like elevations and transparent venation of the youngest leaves (fig. 11). Curly top was transmitted from infected Red Kidney and Lady Washington to Broad Windsor or Horse beans, and from the latter to beets. Four males and six females of twenty specimens survived on Broad Windsor or Horse bean for thirty-six days, when the experiment was discontinued.

SPRING VETCH OR TARE (Vicia sativa), PURPLE VETCH (V. atropurpurea), AND HAIRY, SAND, OR WINTER VETCH (V. villosa)

Spring, purple, and hairy vetch were experimentally infected with curly top. The youngest leaflets nearest the petioles of the compound leaves were often rolled inward along the mid-rib while the terminal leaflets were malformed. The petiole was sometimes bent downward or the petiole and mid-rib showed a spiral twist.
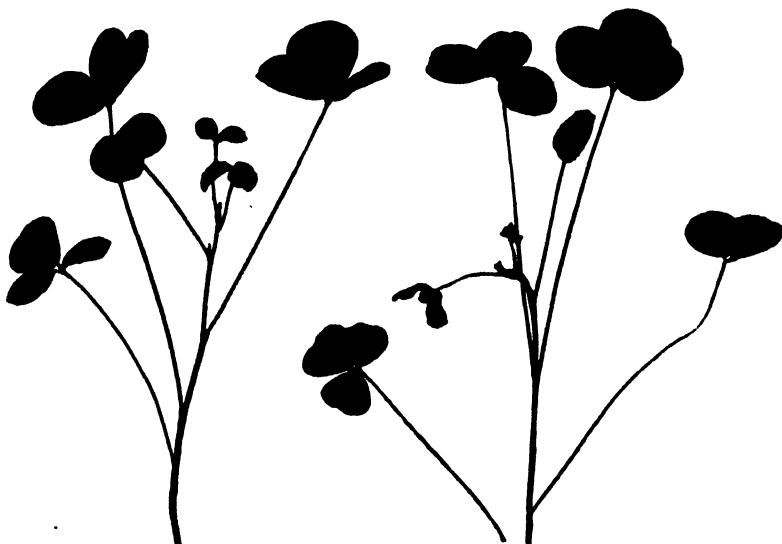
Fig. 13. Shoots of Hairy Peruvian alfalfa (*Medicago sativa*), showing youngest leaves malformed with blister-like elevations.

GARBANZO OR CHICK-PEA (Cicer arietinum)

Garbanzo, or Chick-pea was tested and found to be susceptible to curly top. Marked symptoms of the disease developed. The mid-rib of the youngest compound leaves were distorted and the leaflets were curled inward (fig. 12). In the later stages of the disease, the leaves turned yellow. The males lived from two to five days and the last female died at the end of fourteen days.

HAIRY PERUVIAN ALFALFA (Medicago sativa)

Volunteer Hairy Peruvian alfalfa growing in a beet field near Freeport in the Sacramento Valley was found to be naturally infected with curly top during the 1925 outbreak of the beet leafhopper. Enormous numbers of leafhoppers had congregated on the alfalfa. An examination of the adjacent beet fields planted in March, April, and May showed that many of the beets had died from curly top.

The terrific hot spells during the summer had scorched the outer leaves of the beets, leaving a tuft of diseased, thick, leathery leaves. In all probability, the congregation of the hoppers on volunteer alfalfa was associated with unfavorable food from the badly diseased beets.
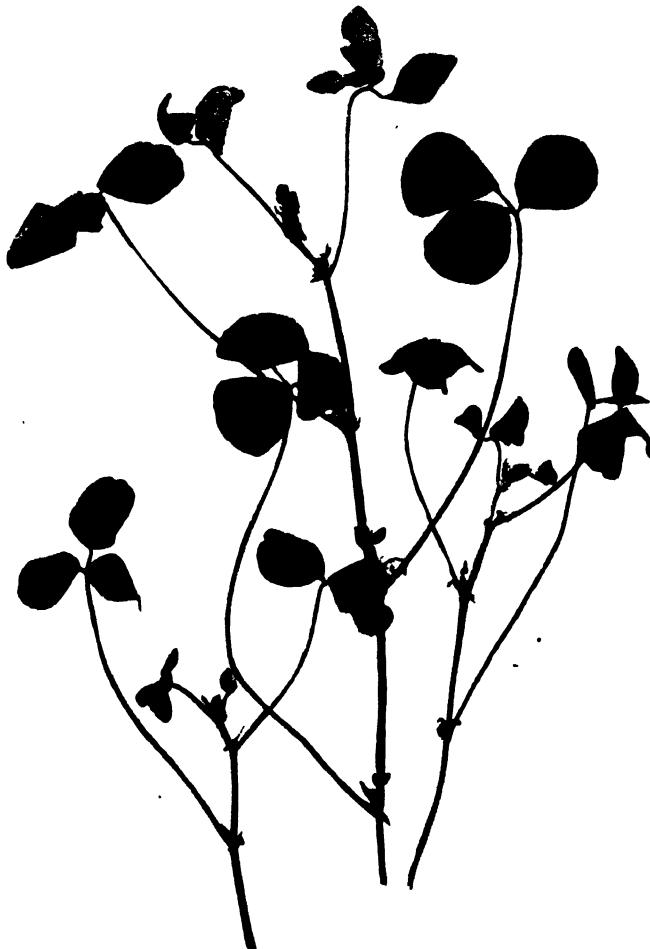
Fig. 14. Stems of Bur clover (*Medicago hispida*), showing three leaflets folded along the sinuous distorted mid-rib.

Hairy Peruvian alfalfa experimentally infected with curly top showed blister-like elevations, and transparent venation on the youngest dwarfed malformed leaves (fig. 13). Six alfalfa plants experimentally inoculated by the infective beet leafhoppers developed curly-top symptoms, while three plants failed to show foliage indications of the disease. Non-infective males did not transmit the disease from alfalfa without curly-top symptoms to beet seedlings.

The males lived from four to six days on young alfalfa and one of five females remained alive for thirty days, while on old alfalfa the last male died at the end of eighteen days and a few females were still alive at the end of thirty-five days, when the experiment was discontinued.
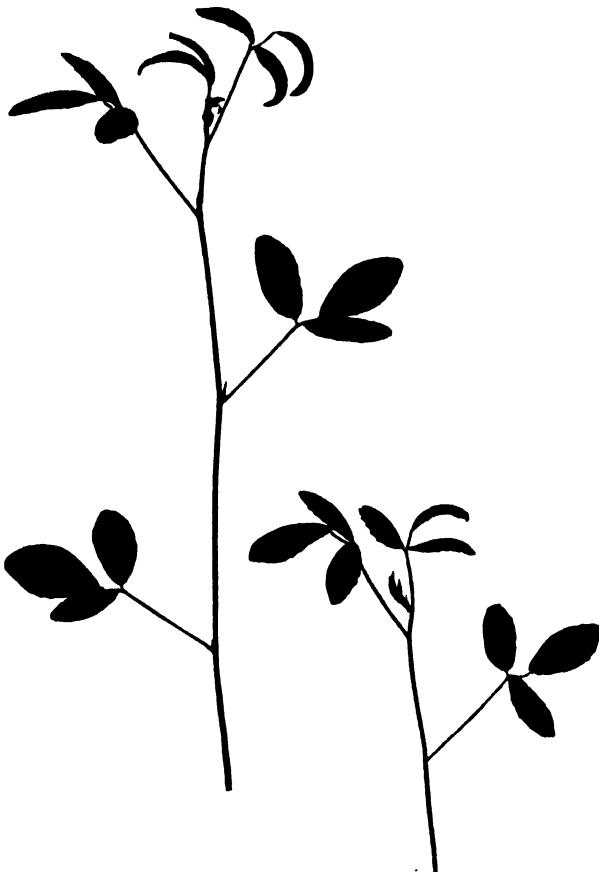
Fig 15. Stems of White Sweet clover (*Melilotus alba*), showing youngest leaves slightly cupped outward.

CLOVERS

Bur clover (*M. hispida*), valued as dry fodder on the plains and foothills in the long rainless summers of California, was tested and found to be susceptible to curly top. In the diseased condition the three leaflets fold along the sinuous distortions of the mid-rib (fig. 14), and transparent venation is evident on the youngest leaves.

White Sweet clover (*Melilotus alba*) was experimentally infected with curly top. The youngest leaflets were cupped outward along the

mid-rib (fig. 15) with faint indications of transparent venation. The males lived from two to seven days and the females from seven to ten days on White Sweet clover.

The three leaflets of Bitter clover (*Melilotus indica*) were rolled toward the petiole (fig. 16) four days after infection with curly top and transparent venation was plainly visible at the end of ten days. The symptoms of curly top in White Dutch clover (*Trifolium repens*), Alsike or Swedish clover (*T. hybridum*), Crimson clover
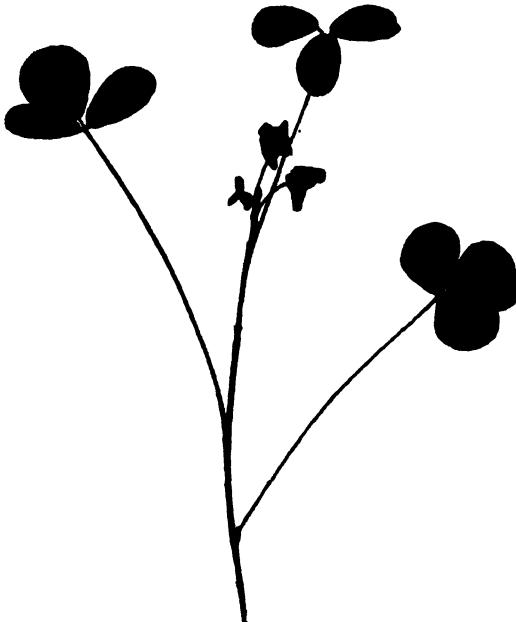
Fig. 16. Tip of plant from Bitter clover (*Melilotus indica*), showing three leaflets rolled toward petiole.

(*T. incarnatum*), Red clover (*T. pratense*), and Mammoth red or Sapling clover (*T. pratense perenne*), experimentally infected, were somewhat similar, the youngest leaflets showed a slight inward roll and transparent venation. In the later stages of the disease the plants turned yellow and died.

LIFE HISTORY OF THE BEET LEAFHOPPER ON LEGUMES

Nymphs which hatched from eggs deposited in the following plants of the Leguminosae completed their life cycle on these host plants in the greenhouse: Broad Windsor or Horse bean; Small Windsor or New Zealand Horse bean; Bell Windsor or Small-seeded Horse bean; Spring vetch; Purple vetch; Hairy Peruvian alfalfa; Bur clover; White Dutch clover; Alsike or Swedish clover, and Red clover.

A

B

Fig. 17.   Delicata squash (*Cucurbita pepo*): *A*, terminal end of runner of naturally infected plant showing puckered and outward-cupped leaves. *B*, malformed, puckered, and cupped leaves removed from the same runner (King City, Salinas Valley, October 9, 1925).

CUCURBITACEAE, GOURD FAMILY

PUMPKIN AND SQUASH (Cucurbita pepo, C. maxima, C. moschata)

Naturally Infected.—The following varieties of pumpkins and squashes growing in the vegetable gardens of the Spreckels ranches near Greenfield and King City in the Salinas Valley were naturally infected with curly top during 1925 and 1926: White Bush Scallop, Summer Crookneck, and Delicata (*Cucurbita pepo*) (fig. 17); Chicago

Fig. 18. Terminal shoots of two plants of Summer Crookneck squash (*Cucurbita pepo*) infected with curly top and of healthy check squash. The cupped leaves are almost globular in shape. Inset shows a flower with calyx but without corolla.

Warted Hubbard (*C. maxima*); Winter Crookneck and Banana (*C. moschata*).

Susceptible Varieties.—The varieties experimentally infected with curly top are California Field, Connecticut Field, Pie Pumpkin, Small Sugar, White Bush Scallop, Yellow Bush Scallop, Summer Crookneck, Vegetable or Italian Marrow, Italian or Zucchine, Long White Vegetable Marrow, Fordhook, Delicata, and Perfect Gem or Cream (varieties of *Cucurbita pepo*); Chicago Warted Hubbard, Golden Hubbard, True Hubbard, Delicious, Boston Marrow, Morse's Marrow (fig. 19), Banana, and Mammoth King (varieties of *C. maxima*); Large Cheese, Green Striped Cushaw, and Mammoth Golden Cushaw (varieties of *C. moschata*).

Symptoms.—A study was made of the development of the symptoms of curly top in the three species of cucurbits grown from seeds, but a considerable amount of variation occurs with reference to the foliage characters of the disease in the different varieties.   As a general rule, the first symptoms to appear were puckering and outward cupping (figs. 17, 18, 19) of the newly developing dwarfed leaves.   In some varieties the cupping continued until the leaves were almost globular in shape (fig. 18).   Transparent venation was often discernible, sometimes accompanied with mottling of the somewhat older leaves.   The dwarfed, cupped leaves and petioles were often dark green, with the stems darker green, compared with healthy plants of

Fig. 19.   Morse's Marrow pumpkin (*Cucurbita maxima*), showing stunted branches with extremely dwarfed leaves.

the same age.   The flowers of infected plants were often dwarfed and dropped from the plants.   The calyx in the larger flowers was present but sometimes no corolla developed (fig. 18, inset).

In some varieties the youngest dwarfed leaves may show a slight cupping or may be normal in shape.   Successive stages of discoloration from mottling of the older leaves to a decided yellowing of the youngest leaves occurred in some varieties (pl. 4, figs. 1, 2).   The yellow discoloration gradually develops between the lateral veins in the older leaves, while the area in the vicinity of the mid-ribs and lateral veins may retain the green color for a time (pl. 4, fig. 2).

Recovery from Disease.—It has been frequently observed in the field that the terminal ends of some runners of naturally infected pumpkins and squashes may show severe curly top symptoms while other runners may be apparently healthy.   Similar observations have

been made with infected pumpkins and squashes grown in the green-
house. In some cases the disease was transmitted to healthy beets
from a shoot showing no symptom on a plant which showed symp-
toms on the stunted portion, while in others it was not. In the case
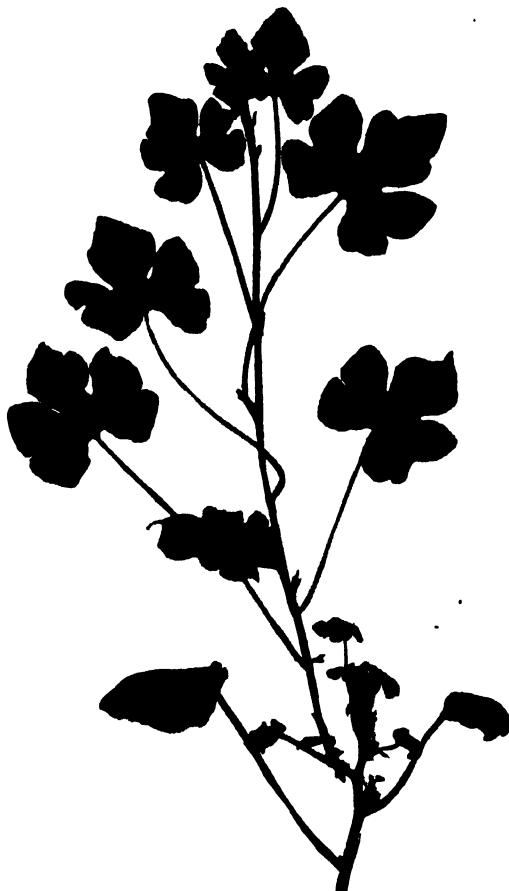of Delicata squash (fig. 20) the disease was transmitted to beets from

Fig. 20. Delicata squash (*Cucurbita pepo*), showing recovery from curly top.
Lower portion of plant shows dwarfed, puckered, cupped leaves, while the
upper shoot shows normal leaves.

the stunted portion of the plant showing puckered, cupped, dwarfed
leaves, but was not transmitted from the shoot with normal leaves.

Longevity of Beet Leafhoppers.—The longevity of the last living
male and female beet leafhopper on different varieties of the three
species of cucurbits grown from seeds is shown in table 2. Spring or
summer-brood adults were used and not the dark overwintering
specimens.

TABLE 2

⁎ Longevity of Last Living Male and Female Beet Leafhopper on
Pumpkins and Squashes

Variety of Pumpkin or Squash	Longevity of males	Longevity of females
Cucurbita pepo	*days*	*days*
California Field	4–5	9
Connecticut Field	4–5	12
Pie	4	10
Small Sugar	5	19
White Bush Scallop	7	14
Yellow Bush Scallop	6	11
Summer Crookneck	10	15
Vegetable or Italian Marrow	2–4	14
Italian or Zucchine	5	10
Long White Vegetable Marrow	4	12
Fordhook	4–5	39
Delicata	6	37
Perfect Gem or Cream	4	21
Cucurbita maxima		
Chicago Warted Hubbard	9	17
Golden Hubbard	3	13
True Hubbard	8	9
Delicious	2–3	14
Boston Marrow	2–3	13
Morse's Marrow	5–6	10
Banana	2–5	11
Mammoth King	4–6	12
Cucurbita moschata		
Large Cheese	5	7
Green Striped Cushaw	5–6	9
Mammoth Golden Cushaw	4–5	5

The adult life of the last living male and female beet leafhopper on varieties of three species of *Cucurbita* may be summarized as follows:

C. pepo: Longevity of males, 2–10 days; females, 9–39 days.

C. maxima: Longevity of males, 2–9 days; females, 9–17 days.

C. moschata: Longevity of males, 4–6 days; females, 5–9 days.

Curly-Top Transmission from One Plant to Another of the Same Variety.—Curly top was transmitted from all of the varieties experimentally infected to other plants of the same variety and then to beets.

Curly-Top Transmission from One Variety to Other Varieties.— The disease was transmitted from one infected variety to different varieties of pumpkins and squashes and then to beets as follows:

> White Bush Scallop to Fordhook to beets.
> Yellow Bush Scallop to Early White Bush Scallop to beets.
> Summer Crookneck to Delicata to beets.
> Vegetable or Italian Marrow to White Bush Scallop to beets.
> Italian or Zucchine to Chicago Warted Hubbard to beets.
> Fordhook to Banana to beets.
> Delicata to White Bush Scallop to beets.
> Perfect Gem (Cream) to Summer Crookneck to beets.
> Chicago Warted Hubbard to Italian (Zucchine) to beets.
> Golden Hubbard to Fordhook to beets.
> Golden Hubbard to Yellow Bush Scallop to beets.
> True Hubbard to Yellow Bush Scallop to beets.
> Delicious to Perfect Gem (Cream) to beets.
> Boston Marrow to Golden Hubbard to beets.
> Morse's Marrow to Connecticut Field to beets.
> Banana to Summer Crookneck to beets.
> Banana to Green Striped Cushaw to beets.

Curly-Top Transmission from Pumpkins and Squashes to Other Crop Plants.—The disease was transmitted from infected pumpkins and squashes to other cultivated plants as follows:

> White Bush Scallop to Ignacia pepper-tomato to beets.
> Banana squash to Novata pepper-tomato to beets.
> Banana squash to Virginia Savoy spinach.
> Mammoth King pumpkin to Arlington White Spine cucumber to beets.

As in the case of beans, curly top is rarely transmitted from infected pumpkins and squashes to the same variety or to different varieties or to other crops by male beet leafhoppers under greenhouse conditions owing to the short life of the males on the three species of *Cucurbita*. Healthy pumpkins and squashes are not favorable host plants of the leafhopper; it is possible that curly top brings about changes in the plant which are of some biological significance to the insect. The beet leafhopper is a sunshine-loving insect; nevertheless, during extremely hot days in the interior regions of California when small favorable weeds wilt, the adults may seek the shade below the large leaves of cucurbits and infect the plants.

Fig. 21.   Watermelon (*Citrullus vulgaris*): *A*, Chilian White Seed showing dwarfed youngest leaves.   *B*, Angeleno showing stunted lateral branches with dwarfed leaves. *C, D, E*, Georgia Rattlesnake showing puckered and cupped leaves.

WATERMELON (CITRULLUS VULGARIS)

During 1926 a patch of watermelons near King City in the Salinas Valley was destroyed by curly top. The varieties proved to be naturally infected with the disease were Klondyke and Excell.

The following varieties were experimentally infected with curly top: Angeleno (fig. 21*B*), Chilian, Black-seeded Chilian, White-seeded

Chilian (fig. 21*A*), Florida Favorite, Georgia Rattlesnake (fig. 21*C*, *D*, *E*), Golden Honey, Kleckley's Sweet, Klondyke, Kolb's Gem, and Tom Watson.

The youngest leaves of the terminal shoots of infected watermelons show a slight puckering and outward curling (fig. 21*B*). The youngest leaves are deep green, in contrast with the yellow of the older leaves.

Fig. 22. Klondyke cucumbers (*Cucumis sativus*): left, check or control plant on which five non-infective males fed; right, plant infected with five infective males, showing stunting. The leaves above the cotyledons show different stages of yellowing.

Naturally infected watermelons were stunted and yellow, with dwarfed leaves at the terminal end of the runners.

The longevity of the beet leafhopper on the susceptible varieties listed was as follows: males, 2–11 days; females, 5–19 days.

CITRON (CITRULLUS VULGARIS)

Red Seeded citron was found to be susceptible to curly top. The foliage symptoms were similar to those in watermelons. The longevity of the males was 2–6 days and females 4–10 days.

CUCUMBER (CUCUMIS SATIVUS)

During 1925 and 1926, the following varieties of cucumbers were demonstrated to be naturally infected with curly top in the Salinas Valley: Early Fortune, Long Green, and a variety either Chicago Pickle or Long Green.

It was shown that the following varieties of cucumbers were susceptible to the disease: Arlington White Spine, Boston Pickling,
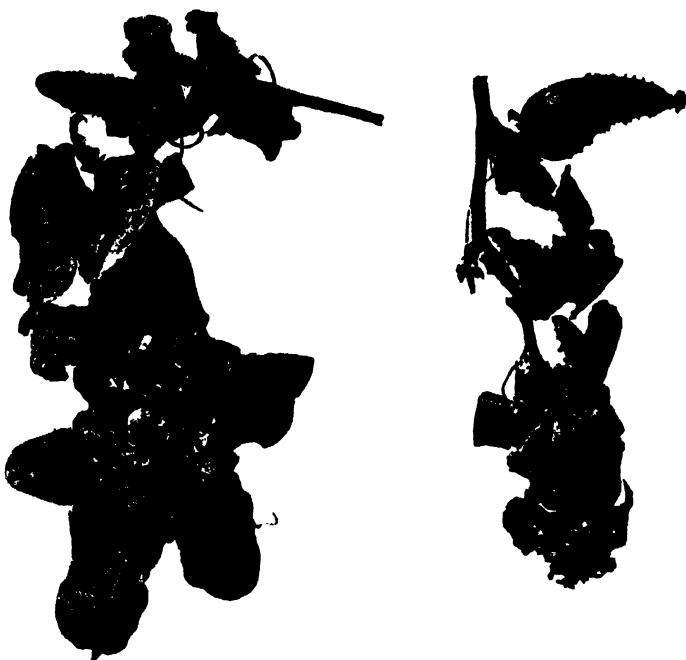
Fig. 23. Long Green cucumbers (*Cucumis sativus*) naturally infected with curly top, showing slightly cupped, densely clustered, dwarfed leaves, and malformed fruit (King City, Salinas Valley, October 9, 1925).

Early Cluster, Early Frame, Improved Boston Pickling, Improved Telegraph, Japanese Climbing, Klondyke, Lemon, Long Green, Snow's Pickling, Telegraph Rollinson's English Greenhouse, and White Spine.

Several varieties of cucumbers infected with curly top after the first true leaves developed were stunted. The youngest leaves became deep green in color, while the older leaves turned yellow. The yellowing begins at the margin of the leaf (fig. 22) and progresses between the lateral veins toward the mid-rib with a green area along the veins, and at the base of the leaf. Later the stem often bends near the surface of the soil, and the plant wilts and dies. The leaves at the

terminal ends of the runners of naturally infected cucumbers were dwarfed, sometimes slightly cupped and densely clustered together (fig. 23). The fruit was dwarfed and often malformed (fig. 23)

The last living males lived from 3 to 40 days, and the females from 7 to 55 days, in varieties of cucumbers susceptible to curly top.

GHERKIN (CUCUMIS ANGURIA)

Six of nine gherkins tested were susceptible to curly top, after the plants were repeatedly inoculated with different lots of infective males. One of the six plants developed transparent venation on the

Fig. 24. Leaves of Golden Lined Rocky Ford muskmelon (*Cucumis melo reticulatus*) infected with curly top, showing dwarfing and slight puckering with margins turned down.

youngest leaf, but five plants failed to show reliable symptoms of the disease. The adult life on gherkins was as follows: males, 3–9 days; females, 7–11 days.

MUSKMELON (CUCUMIS MELO RETICULATUS)

During 1925 and 1926, three varieties of muskmelons were found to be naturally infected with curly top in the Salinas Valley as follows: Green Nutmeg, Pollock, and Tip Top.

The varieties of muskmelons found to be susceptible to the disease are: Acme, Banana, Blenheim Orange, Burrell's Gem, Early Hackensack, Gold Lined Rocky Ford, Green Nutmeg, Honey Ball Melon, Montreal Market, Paul Rose, Persian, Pollock, Rocky Ford, Tip Top, and Windsor Castle.

Varieties of muskmelons experimentally infected with curly top showed no reliable symptoms. The dwarfed youngest leaves of the stunted plants were sometimes puckered with margins slightly turned down (fig. 24). In the later stages of the disease the leaves became yellow, which was also the case with naturally infected muskmelons. The flowers were also dwarfed and often became dry before the petals expanded. In extreme cases of dwarfing, the flowers were reduced to small round knobs.

The males lived from two to six days and the females from seven to sixteen days on the susceptible varieties of muskmelons.

CANTALOUPE (Cucumis melo cantalupensis)

Salmon tint cantaloupe was found to be naturally infected with curly top in the Salinas Vally during 1925. The varieties tested and found to be susceptible to the disease were Large Yellow and Salmon Tint. The symptoms of curly top on cantaloupes were similar to those on muskmelons. The longevity of the beat leafhoppers on the susceptible varieties was as follows: males, 1–4 days; females, 4–10 days.

HONEY DEW MELON (Cucumis melo inodorus)

During 1926, Honey Dew melon (Hybrid Cassaba) was proven to be naturally infected with curly top in the Salinas Valley. It was also experimentally infected with the disease in the greenhouse. The symptoms were similar to those of the disease on muskmelons. The males lived from one to four days and the females from three to nine days on Honey Dew melons.

CASSABA (Cucumis melo inodorus)

Golden Beauty and Winter Pineapple were tested and found to be susceptible to curly top. The symptoms of the disease were similar to muskmelon. The adult life on the two susceptible varieties of cassaba was as follows: males, 1–7 days; females, 2–12 days.

LIFE HISTORY OF BEET LEAFHOPPER ON CUCURBITS

Nymphs which hatched from eggs deposited in the following plants of the Cucurbitaceae completed their life cycle on these host plants in the greenhouse: Early White Bush Scallop squash, and Boston Pickling, Improved Telegraph, and White Spine cucumbers.

RECOMMENDATIONS ON PLANTING TIME OF VARIOUS
CULTIVATED PLANTS TO AVOID CURLY TOP

If the planting schedule as determined for the sugar beet is made use of with mangel wurzel (stock beets), garden beets, Swiss chard, and spinach, better crops will be harvested.

Planting in Natural Breeding Areas.—The following planting schedule of sugar beets in the San Joaquin Valley and the interior regions of the Salinas Valley, usually insures a marketable crop even if a severe outbreak of beet leafhopper occurs. This schedule is related to the spring and autumn dispersal of the insects in natural breeding areas such as these valleys. The fact that in natural breeding grounds most of the insects leave the cultivated areas and fly to the foothills during the autumn has an important bearing on the time of planting beets. Beets should be planted in December, January, and February in the San Joaquin Valley and interior regions of the Salinas Valley. The spring dispersal from the foothills into the cultivated areas usually occurs in April; in some years flights begin in late March and in other years in May. If late spring rains occur, a partial second brood develops and flights may continue in June. If the foliage of sugar beets covers the rows at the time of the invasion of the pest, a good crop can usually be obtained. Early planting, however, is not always safe in the San Joaquin and upper Salinas valleys, as was evident during 1919, when over one-half of the beet crop was blighted by the overwintering hoppers which remained in the cultivated areas.

Planting in Migratory Breeding Areas.—In the Sacramento Valley the overwintering beet leafhoppers are exterminated in the cultivated areas and on the foothills of the Coast Range. There has never been a case of curly top observed in the early-planted beet fields until after the migratory flights occurred. During 1927 and 1928, beets were planted in November but no curly-top beets were found until after the spring migration began. Early planting from November to the end of February insures a crop in the Sacramento Valley during an outbreak of the pest. *During the serious outbreaks of the leafhopper in 1919 and 1925, beets planted in March and April were destroyed* by curly top. In 1925 it was demonstrated that beets planted after the migratory flights ceased in May made a marketable crop. Small migratory flights into the Saramento Valley sometimes occur in April but the large flight usually takes place in May. *In years between*

outbreaks of the pest, beets planted during March and April in the Delta districts usually make good tonnages. If early planting is practiced year after year in the Sacramento Valley, a marketable product will be harvested; on the other hand, if late planting during March and April is adopted year after year, serious losses will be sustained when large migrations of the pest occur.

Planting in Fog Belts.—In the fog belt planting should be discontinued from March first until after the spring flights cease. In the fog belt of the Salinas Valley late plantings in May and June usually result in a good crop. In 1925, however, the late plantings were badly diseased, owing to the fact that a partial second brood developed on the foothills.

Planting Resistant Beans.—After the outbreak of curly top in Small White beans during 1925, the substitution of Pink beans in the Sacramento and Salinas valleys during 1926 was recommended. A large number of Pink beans with slightly puckered leaves were tested from the two valleys but up to the present time not a single plant was found to be naturally infected with curly top.

SUMMARY

The following field and garden plants of three families have been found to be naturally infected with curly top in California:

Chenopodiaceae, Goosefoot or Saltbush family

Sugar Beet (*Beta vulgaris*).

Beta maritima.

Mangel Wurzel or stock beets (*Beta vulgaris*): Giant Yellow, Golden Tankard, Half Sugar, Mammoth Long Red, Red Eckendorf, Yellow Eckendorf, and Sludstrup.

Garden, table, or red beets (*Beta vulgaris*).

Swiss chard (*Beta vulgaris cicla*).

Spinach (*Spinacia oleracea*): Bloomsdale Savoy.

Leguminosae, Pea family

Field and garden beans: Bountiful, Cranberry, Kentucky Wonder, Long Red Kidney, Small White, Stringless Green Pod, and White-seeded Kentucky Wonder (varieties of *Phaseolus vulgaris*); and Baby Lima, or Henderson Bush (*P. lunatus*).

Blackeye cowpea (*Vigna sinensis*).

Alfalfa (*Medicago sativa*): Hairy Peruvian.

Cucurbitaceae, Gourd family

Pumpkins and squashes: Delicata, Summer Crookneck, and White Bush Scallop (*Cucurbita pepo*); Hubbard and Chicago Warted Hubbard (*C. maxima*); Banana, Yellow Summer Crookneck, and Winter Crookneck (*C. moschata*).

Watermelon (*Citrullus vulgaris*): Klondyke and Excell.

Cucumber (*Cucumis sativus*): Early Fortune, Long Green, and a variety either Chicago Pickle or Long Green.

Muskmelon (*Cucumis melo recticulatus*): Green Nutmeg, Pollock, and Tip Top.

Cantaloupe (*Cucumis melo cantalupensis*): Salmon Tint.

The following varieties of economic plants were experimentally infected with sugar beet curly top:

Chenopodiaceae, Goosefoot or Saltbush family

Swiss chard (*Beta vulgaris cicla*): Giant Lucullus, Improved Silver, and Large Ribbed White.

Spinach (*Spinacia oleracea*): Bloomsdale Savoy, Long Standing, Round Summer, Prickly Seeded, New Zealand, and Virginia Savoy.

Leguminosae, Pea family.

Field beans (*Phaseolus vulgaris*): Bayo, Blue Pod, Cranberry, Lady Washington, Pink, Red Kidney, Red Mexican, Small White, and Spotted Red Mexican.

Limas (*P. lunatus*): Burpee's Bush, Fordhook Bush, Lewis, and Baby Lima or Henderson Bush.

Garden beans: Bountiful, Early Refugee, Golden Wax, Kentucky Wonder Pole, Kentucky Wonder Wax, Prolific Black Wax, Scarlet Runner Pole, Stringless Green Pod, White Creaseback, and White-seeded Kentucky Wonder.

Cowpeas (*Vigna sinensis*): Blackeye and Whippoorwill or Speckled.

Horse beans (*Vicia faba*): Broad Windsor or Horse bean, Small Windsor or New Zealand Horse bean, and Bell Windsor or Small-seeded Horse bean.

Spring vetch (*Vicia sativa*), Purple vetch (*V. atropurpurea*), and Hairy sand or winter vetch (*V. villosa*).

Garbanzo or Chick-pea (*Cicer arietinum*).

Hairy Peruvian alfalfa (*Medicago sativa*).

Bur clover (*M. hispida*), White Sweet clover (*Melilotus alba*), Bitter clover (*M. indica*), White Dutch clover (*Trifolum repens*), Alsike or Swedish clover (*T. hybridum*), Crimson clover (*T. incarnatum*), Red Clover (*T. pratense*), and Mammoth Red or Sapling clover (*T. pratense perenne*).

Curcurbitacea, Gourd family.

Pumpkins and squashes: California Field, Connecticut Field, Pie Pumpkin, Small Sugar, White Bush Scallop, Yellow Bush Scallop, Summer Crookneck, Vegetable or Italian Marrow, Italian or Zucchine, Long White Vegetable Marrow, Fordhook, Delicata and Perfect Gem or Cream (varieties of *Cucurbita pepo*); Chicago Warted Hubbard, Golden Hubbard, True Hubbard, Delicious, Boston Marrow, Morse's Marrow, Banana, and Mammoth King (varieties of *C. maxima*); Large Cheese, Green Striped Cushaw, and Mammoth Golden Cushaw (varieties of *C. moschata*).

Watermelons (*Citrullus vulgaris*): Angeleno, Chilian, Black-seeded Chilian, White-seeded Chilian, Florida Favorite, Georgia Rattlesnake, Golden Honey, Kleckley's Sweet, Klondyke, Kolb's Gem, and Tom Watson.

Citron (*C. vulgaris*): Red Seeded.

Cucumbers (*Cucumis sativus*): Arlington White Spine, Boston Pickling, Early Cluster, Early Frame, Improved Boston Pickling, Improved Telegraph, Japanese Climbing, Klondyke, Lemon, Long Green, Snow's Pickling, Telegraph Rollinson's English Greenhouse, and White Spine.

Gherkin (*C. anguria*).

Muskmelons (*C. melo reticulatus*): Acme, Banana Blenheim Orange, Burrell's Gem, Early Hackensack, Gold Lined Rocky Ford, Green Nutmeg, Honey Ball Melon, Montreal Market, Paul Rose, Persian, Pollock, Rocky Ford, Tip Top, and Windsor Castle.

Cantaloupes (*C. melo cantalupensis*): Large Yellow and Salmon Tint.

Honey Dew melon or Hybrid Cassaba (*C. melo inodorus*).

Cassaba (*C. melo inodorus*): Golden Beauty and Winter Pineapple.

ACKNOWLEDGMENT

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PLATES 1-4

PLATE 1

Sugar Beet (*Beta vulgaris*)

Fig. 1. Beet leaves showing blister-like elevations.

Fig. 2. Portion of beet leaf showing blister-like elevations magnified.

Fig. 3. Beet leaves showing blister-like elevations and transparent venation.
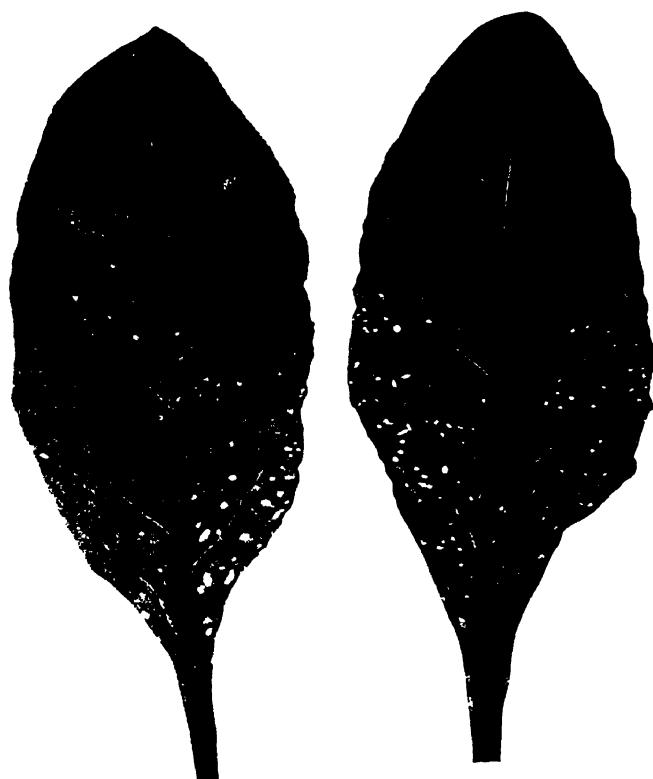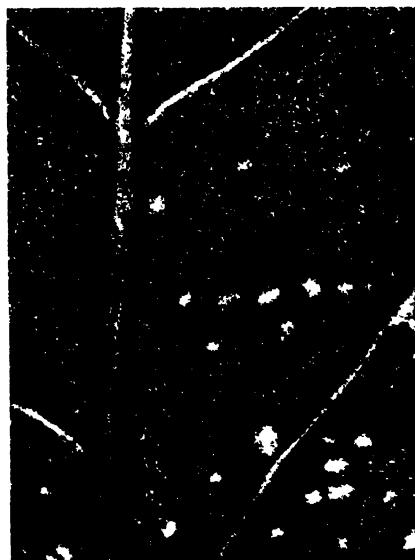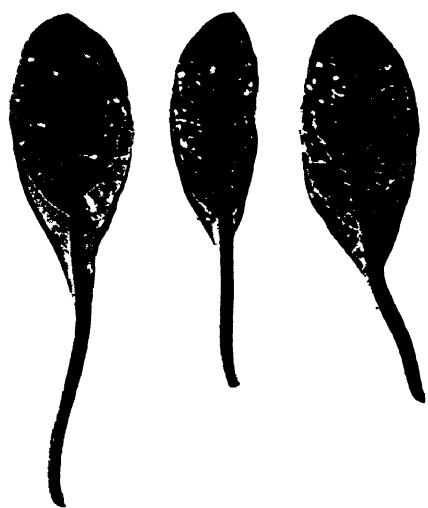
Fig. 1

Fig. 2

Fig. 3

PLATE 2

Sugar Beet (*Beta vulgaris*)

Fig. 1.   Beet leaf showing normal venation.

Fig. 2.   Beet leaf showing the transparent network of minute veins usually present on the youngest leaves of curly-top beets.
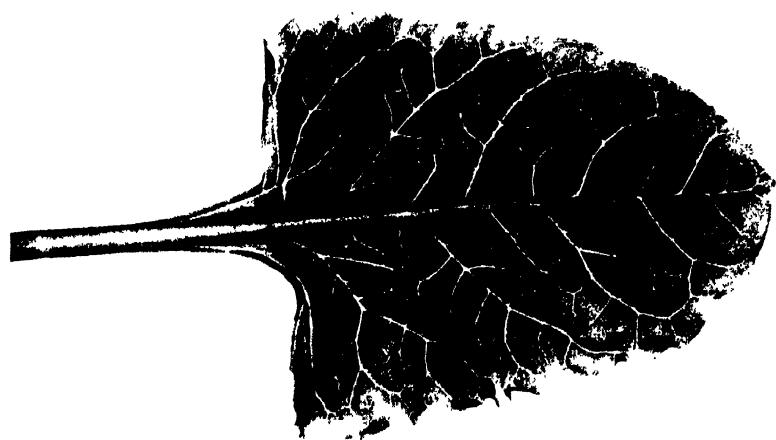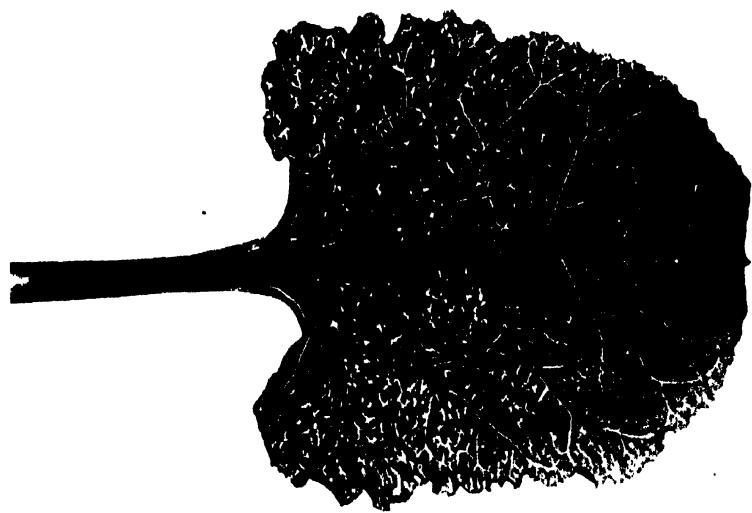
Fig. 1

Fig. 2

PLATE 3

Sugar Beet (*Beta vulgaris*)

Fig. 1. Leaf from curly-top beet showing small wart-like elevations on the veins, giving the lower surface of the blade a roughened appearance.

Fig. 2. Small wart-like protuberances limited to the lower right side of the beet leaf.

Fig. 3. Leaf from curly-top beet showing nipple-like papillae and knot like swellings on the distorted veins.

Fig. 4. Leaves from curly-top beet showing black liquid exudation on petioles and veins.

Fig. 1

Fig. 2

Fig. 3

Fig. 4
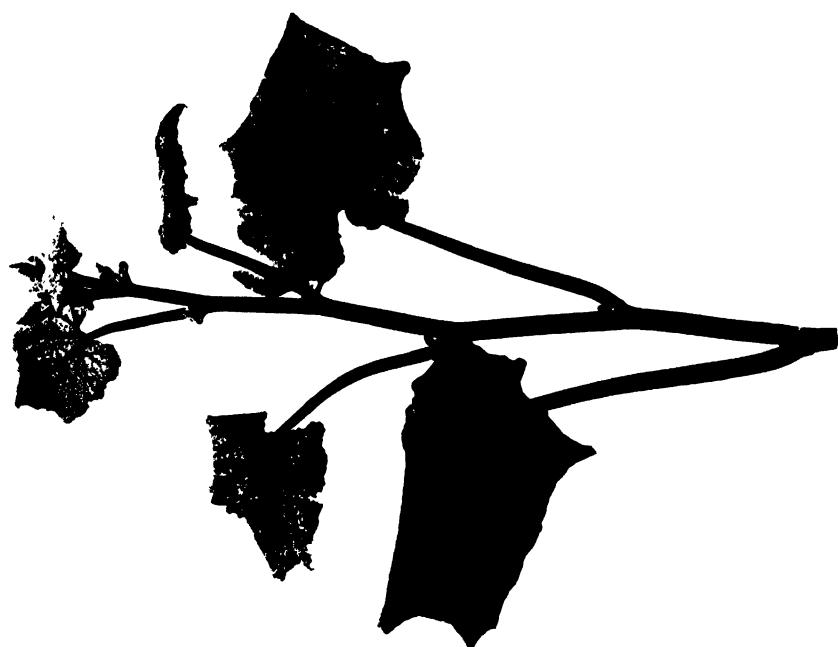
Fig. 1

Fig. 2

THE CHEMICAL EFFECT OF GYPSUM, SULFUR, IRON SULFATE, AND ALUM ON ALKALI SOIL[1]

W. P. KELLEY[2] AND ALEXANDER ARANY[3]

INTRODUCTION

Until recently the toxicity of alkali soils was attributed almost exclusively to the presence of an excess of one or more of the soluble salts, and the general opinion has been that the toxic conditions will be removed by leaching out the soluble salts. Where sodium carbonate occurs Hilgard pointed out that it may be necessary to apply some substance, such as gypsum, which will convert the carbonate into a neutral salt; otherwise it may be difficult or even impossible to leach out the soluble salts, owing to the deflocculated condition of the soil that is produced by alkali carbonates. In any case the prevailing opinion has been that the removal of the soluble salts will overcome the toxic conditions. Numerous attempts have been made to reclaim alkali soils by applying this idea. In some instances good success has been obtained; in others the results have been disappointing.

Investigations on the base-exchange phenomenon during the past fifteen years have thrown important light on this question. Whereas the older idea presupposed that the soluble salts are commingled with and form a mere mechanical mixture with the insoluble constituents of the soil, these base-exchange investigations have shown that under certain conditions the soluble salts produce substantial chemical changes in certain components of the clay constituents of the soil, and that these changes are as important as and even more difficult to overcome than the soluble salts. Ordinary leaching may remove the latter, but the leaching process will not bring about the needed chemical change in the former.

The inorganic base-exchange constituents of non-alkali soils, sometimes referred to as zeolites, are chiefly calcium and magnesium compounds, with the former usually present in much the greater amount. On the other hand, sodium compounds predominate among the soluble salts of alkali soils; not infrequently the soluble salts are composed almost entirely of sodium compounds. As the sodium salts accumulate during the course of alkali-soil formation, the sodium reacts with the base-exchange constituents of the soil, replacing more or less calcium and magnesium and forming simple salts of these bases and sodium-exchange compounds. We now know that alkali soil which contains an excess of replaceable sodium is likely to be more or less toxic to plants even after the excess of soluble salts has been leached out.

It follows, therefore, that in addition to the removal of the excess of soluble salts, the successful reclamation of an alkali soil may necessitate the displacement of a part, at least, of the replaceable sodium. In fact the reclamation will probably not be truly complete until practically all of the exchangeable sodium has been replaced, although, as will be shown later, satisfactory crops may be grown when sodium does not comprise too high a percentage of the total replaceable bases.

Where neutral sodium salts accumulate, the exchange of bases rarely, if ever, goes to completion, owing to the nature of the equilibrium. On the other hand, if carbonate is a constituent of the soluble salts, as is often the case in black-alkali soils, the replaced calcium will be precipitated as the carbonate, and the magnesium probably as the basic carbonate, which precipitation will materially affect the equilibrium. In this case, especially if the total concentration of sodium salts is high, a large percentage of the base-exchange constituents will be converted into sodium compounds and the replaced

calcium and magnesium will be precipitated in the soil as carbonates. If potassium is a constituent of the accumulated soluble salts, this element will also participate in the base-exchange reactions with the formation of more or less of potassium-exchange compounds. The presence of soluble calcium, on the other hand, materially affects the replacement of soil bases by sodium salts, and if present in sufficient concentration, soluble calcium may prevent the replacement of calcium from the exchange complex even though the total concentration of sodium salts is high.[4]

On the basis of the results of studies on the general principles of base exchange,[6, 8, 16] and of the study of several types of alkali soils, Kelley and Brown[13] pointed out that a determination of (a) the water-soluble cations, especially calcium and sodium, (b) the replaceable bases, (c) the OH-ion concentration of the soil and (d) the composition of the irrigation water together make it possible to predict whether leaching alone will bring about successful reclamation.

Whether the exchange complex has been only partially or wholly converted into sodium compounds through the exchange of bases, this complex is not removed from the soil by ordinary leaching with water; neither will moderate leaching convert these sodium compounds into calcium compounds except under special conditions and then the conversion will usually be only partial. It follows, therefore, that the mere leaching and drainage of an alkali soil may not produce satisfactory reclamation.

In connection with field experiments, which the University of California has been conducting for several years on alkali soil near Fresno, California, different materials have been applied. Some of these materials have produced marked improvement in the growth and yield of crops, whereas leaching with water without other treatment has not produced satisfactory results. Since previous investigations[12, 13] have shown that sodium comprises a relatively high percentage of the total replaceable bases of this soil and that there is an excess of soluble sodium salts present, including sodium carbonate, it is a matter of special interest to study the chemical changes that

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[4] In this paper it is assumed that, apart from the soluble salts, an alkali soil represents materials which once were similar to those of a non-alkali soil of the same type, but which have since been acted on by soluble salts. Whether the soluble salts acted on the soil materials before or after the latter were laid down in their present location is perhaps inconsequential, in so far as this discussion is concerned. As a matter of fact the sodium salts, including the carbonate, accumulated in the area under consideration some time later than 1890 in consequence of seepage and poor drainage. Before that time this soil was free from an excess of salts and produced successful crops of various kinds.

have been brought about in the soil both as regards the replaceable bases and the soluble salts, and to attempt to correlate these changes with the crop responses. A description of the field experiments, including a record of the treatments and the crop yields, is given in a separate paper.[18] The present paper will be devoted to a consideration of the chemical changes that have been produced in the soil.

The following plots have been investigated: Plot 4, treated in 1920 with 9 tons per acre of gypsum and again in 1921 with 6 tons per acre of gypsum; plot 10, treated in 1921 with 3600 pounds per acre of finely ground elemental sulfur; plot 11, treated in 1921 with 10 tons per acre of gypsum; plot 17, treated in 1922 with 9 tons per acre of ferrous sulfate; plot 18, treated in 1923 with 10 tons per acre of potassium alum; plot 19, treated in 1922 with 20 tons per acre of stable manure and in 1925 with 1000 pounds per acre of elemental sulfur, and plot 45, treated in 1925 with 5 tons per acre of ferrous sulfate.

Each of these plots was sampled before the materials were applied and again after the lapse of two or more years. The samples (14 or more) were taken at intervals of ten feet across the greatest diameter of each plot. In the interval between the sampling dates the plots were heavily flooded at certain times in order to leach out the soluble salts and to assist in making the treatments effective. All of the plots have been irrigated freely whenever any crop was being grown. These samples have been analyzed individually for soluble salts and the results are being reported elsewhere.[18] Recently a series of composite samples were prepared by mixing, for each such sample, equal quantities of the individual samples that were drawn from a given plot, and these composites were used in the present study.

It should be pointed out in this connection that when the plot experiments were first begun the content of soluble salts was by no means the maximum concentration that this soil had previously contained. The quarter section (160 acres), of which these experimental plots comprise only a part, was flooded and kept covered with water for a period of two or more months in the summer of 1914 and again in 1915,[21] in what has proved to be an unsuccessful attempt to reclaim the land by flooding and drainage. Although the total concentration of soluble salts in the various parts of this area before it was flooded is not known definitely, it is certain that very much of the salts was then leached out. When the plot experiments were begun the soil was extremely toxic to plants, previous plantings of barley and alfalfa having failed to germinate over a large part of the experimental area.

The soil is a fine sandy loam the upper horizon of which contains a small but variable amount of calcium carbonate. The texture is practically uniform down to a depth of two or more feet, where a compacted layer resembling hardpan is found. This layer, the thickness of which ranges from two to six or more inches, contains considerable insoluble carbonate (chiefly calcium carbonate). This layer is hard and difficult to penetrate when dry, but water readily passes through it, and when it is wet the soil auger cuts through without difficulty. Beneath this calcareous layer many similar layers, separated from each other by soil and clay materials, occur down to a depth of fifty or more feet. In certain places the uppermost calcareous layer occurs at a depth of about two feet, while a few inches away it may be three or four feet below the surface. Because of this fact the two sets of soil samples from certain of the experimental plots, especially those representing the third and fourth feet in depth, were found to vary considerably in their content of insoluble carbonate.

The methods used in this study were those which have been elaborated and employed in the laboratory of the Citrus Experiment Station for several years. The samples were analyzed for water-soluble constituents, total carbonate, replaceable bases and hydrogen-ion concentration. The soluble constituents were determined by shaking 200 grams of soil with 1000 cc. of distilled water for one hour, filtering and analyzing the filtrate. Total carbonate was determined gravimetrically by boiling 10 grams of soil with dilute sulfuric acid and absorbing the CO_2 in potash bulbs. The replaceable bases were determined by a modification of the ammonium chlorid method, which is discussed in another paper,[15] and the pH values were determined on a suspension of the soil by means of the hydrogen electrode.

EFFECT ON THE SOLUBLE SALTS

In agreement with the results of previous studies on this area [11], the analyses presented in tables 1–7 show that the initial distribution of water-soluble salts was variable in these plots. This is especially true as regards the normal carbonate, the concentration of which was originally the highest in the surface soil of each plot. With plots 4, 10 and 11 considerable soluble carbonate was found down to a depth of four feet, while below the depth of two feet plots 18 and 45 contained much less soluble carbonate and the same was true of the fourth foot of plots 17 and 19. It was only in the case of the third and fourth feet of plot 18 that the original samples contained any appreciable amount of soluble calcium and magnesium.

The data show that the content of water-soluble salts in the upper horizon of each plot has been materially reduced by each of the treatments. However, certain soluble constituents, notably sodium, sulfate and carbonate, have been increased in the third and fourth feet of certain plots. This is due chiefly to the leaching down of soluble substances from the upper layers. Those plots which were most thoroughly leached (plots 4, 10 and 11) have been almost completely freed from chlorid down to the depth of at least four feet, whereas considerable chlorid still remains in the deeper layers of the other plots. For example, in the case of plots 19 and 45, which have been leached only moderately, chlorid has markedly accumulated in the fourth foot. In the case of the first foot of plots 4, 10 and 11, the treatments have produced notable increases in the content of soluble calcium and magnesium.

TABLE 1

PLOT 4, TREATED WITH 15 TONS GYPSUM PER ACRE;

WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0–12 inches		12–24 inches		24–36 inches		36–48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO_3	1.04	tr	0.71	0.35	0.66 ˉ	0.36	0.56	0.43
HCO_3	1.11	0.38	1.04	0.61	0.79	0.56	0.71	0.62
Cl	0.14	0.06	0 31	0	0 33	0	0 27	0
SO_4	0.73	0.39	0 55	0 24	0.32	0 25	0.25	0 25
Ca	tr	0.47	tr	tr	tr	tr	tr	tr
Mg	tr	0.25	tr	tr	tr	tr	tr	tr
K	0	0	0	0	0	0	0	0
Na	3.01	0.12	2.61	1.19	2.10	1.18	1.77	1.29

The chlorid determinations, when considered in connection with the amount of water that has been applied, show that it is possible to remove the soluble neutral salts from this soil by leaching. It is even possible, as shown by special leaching experiments, to remove practically all of the soluble carbonate. On the other hand, moderate leaching may actually increase the amount of soluble carbonate. This, as was shown by Gedroiz[7] and others[13] is due chiefly to the fact that, upon lowering the concentration of soluble sodium, calcium carbonate reacts with the sodium-exchange complex, forming calcium-exchange complex and sodium carbonate. This reaction is promoted

by carbon dioxid because of its influence on the OH-ion concentration and its solvent power for calcium carbonate. If an abundance of carbon dioxid is present, the soluble end-product of this reaction will be bicarbonate rather than the normal carbonate. This effect of calcium carbonate and carbon dioxid is a very important one and should be utilized to the fullest extent possible in the reclamation of black-alkali soil. This question will be discussed at greater length in a separate paper that will be devoted to a consideration of the effects of leaching as such.

TABLE 2

PLOT 10, TREATED WITH 3600 POUNDS SULFUR PER ACRE;

WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0–12 inches		12–24 inches		24–36 inches		36–48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO_3	1.14	0.00	0 54	0.00	0 66	0 60	0 34	0 93
HCO_3	1.60	0.75	1 04	0 75	1.23	1 03	1 00	1.14
Cl	1.53	0.00	1 20	0.00	0 68	tr	0 53	0 23
SO_4	0.99	0 44	0.53	0.45	0.15	0.64	0 08	0 62
Ca	tr	0.46	tr	tr	0	0	0	0
Mg	tr	0.35	tr	tr	0	0	0	0
K	0.02	0 00	0	0	0	0	0	0
Na	5.24	0.38	3.30	1 19	2.72	2 26	1.94	2 91

TABLE 3

PLOT 11, TREATED WITH 10 TONS GYPSUM PER ACRE;

WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0–12 inches		12–24 inches		24–36 inches		36–48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO_3	1.19	tr	0.89	0.69	0.71	1.34	0.40	1.27
HCO_3	1.23	0.62	0.97	0.98	1.01	1.03	0.78	1.09
Cl	1.33	0.15	1.08	0	0.78	0	0.59	0 20
SO_4	0.20	0.43	0.21	0.39	0.18	0.24	0.15	0.26
Ca	tr	0.19	tr	tr	tr	tr	tr	tr
Mg	tr	0.22	tr	tr	tr	tr	tr	tr
K	0.05	0	0	0	0	0	0	0
Na	3.90	0.86	3.14	1.97	2.67	2.61	1.92	2.81

It will be noted that in the case of plot 10, which was treated in 1921 with 3600 pounds of sulfur per acre, there has been a complete removal of soluble carbonate to a depth of two feet. On the other hand, plot 19, which was treated in 1922 with 20 tons per acre of manure and in 1925 with only 1000 pounds per acre of sulfur, has not been so completely freed from soluble carbonate. Each of these treatments has caused a slight increase in the soluble carbonate in the deeper subsoil. This, as suggested above, was probably due chiefly to the leaching down of carbonates from the upper layers. It is especially interesting to see that no appreciable increase in soluble carbonate has taken place in the subsoil of the plot that was treated with a large amount of a substance, which converts soluble carbonate into insoluble calcium carbonate (gypsum, plot 4), or which decomposes carbonate (iron sulfate, plot 17, and alum, plot 18). On the other hand, the subsoil of those plots which were treated with a smaller amount of these materials (plot 11, treated with 10 tons per acre of gypsum, and plot 45, treated with 5 tons per acre of iron sulfate) has sustained an increase in soluble carbonate. It seems that these last named amounts of gypsum and iron sulfate were insufficient to precipitate or decompose all of the soluble carbonate in the upper layers of this soil. As was shown previously,[17, 20] gypsum and iron sulfate react on the soluble carbonate and the sodium-exchange compounds simultaneously. The consequence is that the amount of these materials that must be applied is not determined solely and in some cases not even chiefly by the content of soluble carbonate.[5]

The data strongly suggest that where sulfur is applied to an alkali soil in which the soluble carbonate is largely concentrated near the surface, excessive leaching should be delayed until active oxidation has taken place. In this event the oxidation products will decompose the soluble carbonate, whereas heavy leaching during the earlier stages of the oxidation will tend to wash more or less of the soluble carbonate into the subsoil, just as has been the case in certain of these plots.

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[5] In this connection it is of interest to note that Burgess[4] and his colleagues[2, 3] have concluded from their studies on Arizona alkali soils that sodium carbonate is non-existent in black alkali soils in general. They attribute the alkalinity of this type of soil to hydrolysis of the sodium-exchange complex, rather than to sodium carbonate. Although the sodium complex does undergo more or less hydrolysis, as was discussed in detail several years ago by Gedroiz,[7] Cummins and Kelley[6] and others, there is abundant evidence that sodium carbonate actually occurs in certain black-alkali soils. For example, the displaced soil solution of a sample drawn from an unleached portion of the Fresno experimental area was found to contain 2250 p.p.m. CO_3, and its pH was 9.11. It is evident that this soil must contain normal carbonate.

Soluble carbonate produces a condition in soils which is extremely toxic to plants and this substance should be completely removed from the soil if possible.   If sodium carbonate is leached down into the subsoil, capillarity may cause it to rise toward the surface at some later time.   Moreover, the accumulation of soluble carbonate in the subsoil tends to prevent the development of a deep root system by plants.

TABLE 4

PLOT 17, TREATED WITH 9 TONS FeSO₄ PER ACRE;

WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0–12 inches		12–24 inches		24–36 inches		36–48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO₃	1 38	0 00	0 50	0 22	0 30	0 30	0.11	0 10
HCO₃	1 43	0.95	0 88	0.97	0 76	0 97	0 68	0 85
Cl	1 11	0 13	0.59	0.21	0 50	0 45	0.31	0.40
SO₄	0.57	0.39	0 33	0 32	0 25	0.60	0.18	0 68
Ca	tr	0	0	0	0	tr	0	0 18
Mg	tr	0	0	0	0	tr	0	0 34
K	0	0	0	0	0	0	0	0
Na	4 49	1.48	2 29	1.71	1.80	2 31	1 27	1 51

TABLE 5

PLOT 18, TREATED WITH 10 TONS ALUM PER ACRE;

WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0–12 inches		12–24 inches		24–36 inches		36–48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO₃	1.30	0.00	0.40	0.05	0.32	0.08	0.21	0.00
HCO₃	1.34	0.88	0.98	0.85	0.96	0 86	0 93	0.77
Cl	0.83	0.15	0.51	0.24	0.51	0.48	0.47	0.34
SO₄	0 49	0.34	0.30	0.58	0.49	1 04	0.54	1.17
Ca	0	0	0	0	0.24	0.25	0.18	0.38
Mg	0	0	0	0	0.21	0.36	0.17	0.39
K	0	0	0	0	0	0	0	0
Na	3.96	1.36	2 18	1.70	1.89	1.84	1.85	1.51

TABLE 6

PLOT 19, TREATED WITH 20 TONS MANURE PER ACRE AND 1000 POUNDS SULFUR
PER ACRE; WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0-12 inches		12-24 inches		24-36 inches		36-48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO_3	1 15	0 33	0.50	0 72	0.23	0.36	0.03	0.13
HCO_3	1.72	0 87	1 12	0 99	1.01	0.84	1.01	0.74
Cl..........	1 29	0 18	0 76	0 41	0.60	0.95	0.93	1.02
SO_4	0.64	0 62	0.19	0.82	0.20	1.14	0.23	0.97
Ca	tr	0	tr	tr	tr	tr	tr	0 32
Mg	tr	0	tr	tr	tr	tr	tr	0.46
K	0 07	0	0.03	0	0	0	0	0
Na	4 73	2 00	2 53	2.92	2.04	3 29	2 19	2.08

TABLE 7

PLOT 45, TREATED WITH 5 TONS $FeSO_4$ PER ACRE;
WATER-SOLUBLE CONSTITUENTS

(Milliequivalents per 100 grams.)

	0-12 inches		12-24 inches		24-36 inches		36-48 inches	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
CO_3	0.58	0.10	0.29	0 52	0 10	0.51	0.03	0.10
HCO_3........	1.32	0.80	1.01	0.95	0.79	0.95	0.80	0.63
Cl........	1 46	0.15	0.60	0.20	0.48	0.38	0.43	0.90
SO_4	0.38	0.18	0.13	0.17	0.06	0.75	0.10	0.79
Ca........	tr	0	tr	0	tr	tr	tr	0.34
Mg........	tr	0	tr	0	tr	tr	tr	0.50
K	0.03	0	0	0	0	0	0	0
Na........	3.70	1.23	2.03	1.83	1.44	2.58	1.35	1.56

EFFECT ON THE INSOLUBLE CARBONATE

The data reported in table 8 show that the original carbonate content of plot 4 was approximately the same throughout the four feet in depth, whereas the third and fourth feet of the other plots contained much more carbonate than the first and second feet. It will be noted that the application of gypsum (plots 4 and 11) has produced an increase in the insoluble carbonate of the first foot, while the application of sulfur (plots 10 and 19), iron sulfate (plots 17 and 45) and alum (plot 18) has caused a decrease. In the case of plot 10, which was treated with a relatively large amount of sulfur, and of plot 18, treated with a heavy application of alum, the insoluble carbonate of the second foot has also sustained some loss. On the other hand, it is improbable that any of these treatments have materially affected the insoluble carbonate of the third and fourth feet. It is probable that the differences in the amount of insoluble carbonate found in the two sets of samples representing the third and fourth feet of a given plot were not produced by the treatments, but were due to the inherent variability of the subsoil. This variation seems to be closely related to the sporadic fluctuation in the thickness of and depth to the compacted calcareous layers previously mentioned.

TABLE 8

EFFECT ON THE CONTENT OF INSOLUBLE CO_2

(Milliequivalents per 100 grams.)

Depth in inches	Plot 4		Plot 10		Plot 11		Plot 17	
	Before treatment	After treatment with gypsum	Before treatment	After treatment with sulfur	Before treatment	After treatment with gypsum	Before treatment	After treatment with iron sulfate
0-12	5.49	6 33	4 09	1.22	8.20	8.82	4.68	3.18
12-24	4.01	4.11	2.97	2 25	6.81	6.91	3.72	3.67
24-36	5.19	4.19	21.29	21.47	25 46	18 22	33 01	38.10
36-48	3.40	3.06	32.34	33.43	35.56	35 33	46.70	33.65

Depth in inches	Plot 18		Plot 19		Plot 45	
	Before treatment	After treatment with alum	Before treatment	After treatment with sulfur	Before treatment	After treatment with iron sulfate
0-12	8.33	5.43	5.70	3.66	4 73	3 21
12-24	8.96	8.44	3.85	3.84	4.63	4.62
24-36	52.69	67.00	18.07	23.07	14 69	15.50
36-48	64.06	43 79	46.70	44.05	20.56	31.57

The effects of the several treatments on the insoluble carbonates are in close agreement with the theoretical probabilities that have been discussed in previous publications.[13, 17, 20] For example, gypsum reacts with the soluble carbonate to form calcium carbonate, thus bringing about an increase in the content of insoluble carbonate. On the other hand, sulfur becomes oxidized to sulfuric acid, and both iron sulfate and alum are acidic compounds by virtue of their hydrolytic nature. The consequence is that the application of these materials brings about a conversion of both the soluble and insoluble carbonates into bicarbonate and sulfate, or else a decomposition of carbonates with the consequent evolution of carbon dioxid. When acidic types of material, such as sulfur, iron sulfate, etc., are applied, the resulting reactions bring more or less calcium carbonate into solution and therefore increase the content of soluble calcium. This is an extremely important step in the reclamation process as has been pointed out in previous publications.[13, 17] In fact the practical value of these materials rests in considerable part on their action upon calcium carbonate, as will be more fully discussed later.

EFFECT ON THE REPLACEABLE BASES

As is well known the solubility factor complicates the determination of the replaceable bases in any soil. If the total content of replaceable bases is low and if certain other types of calcium and magnesium compounds are present, the amount of calcium and magnesium that is dissolved by the salt solution used in the determination of the replaceable bases may exceed the total quantity of bases that is actually replaced by the base of the salt solution. This fact is especially important in the study of certain types of alkali soil. Calcium carbonate is a common constituent of black-alkali soils, and, under certain circumstances, normal magnesium carbonate may also be present. Moreover, as was pointed out previously,[13] it is possible that the basic carbonate of magnesium may be formed as a result of base exchange, if soluble carbonate is present. As is well known these carbonates are distinctly soluble in ammonium chlorid, the salt that is commonly used in the determination of the replaceable bases. Gedroiz pointed out[9] that the error in the determination of replaceable calcium caused by calcium carbonate can be calculated from the determination of the total carbonate before and after extracting the replaceable bases. Obviously it is permissible to introduce this correction only where calcium carbonate is the only carbonate present.

In addition to the various carbonates, previously published data[13] strongly indicate that black-alkali soils may contain appreciable amounts of calcium or magnesium silicates which become soluble in the course of the determination of the replaceable bases. Where a mixture of these various carbonates and silicates occurs, we know of no method by which it is possible to make an accurate determination of the replaceable calcium or magnesium.

However, it is reasonably certain that the exchange of ions which takes place between a salt solution and the exchange complex of the soil is a stoichiometric process. For each ion that is replaced from the soil by a given treatment a chemical equivalent of some other ion must be absorbed by the soil. Therefore, the amount of base that a soil absorbs from the neutral salt solution that is used in the determination of the replaceable bases must be equivalent to the total quantity of ions that is replaced by the base of that salt. If the soil is neutral or alkaline, its exchangeable cations are confined chiefly to the replaceable bases. Hence, by thoroughly leaching the soil with a salt solution, whose base was not originally present in the soil and does not form insoluble compounds with the constituents of the soil other than the exchange components,[6] and then by making a determination of the amount of this base that is absorbed, we have an indirect method for the determination of the total content (S) of replaceable bases. The quantity thus found, expressed as chemical equivalents, less the sum of the replaceable potassium and sodium, gives a measure of the replaceable divalent bases. Using this method of calculation Kelley and Brown[13] concluded that certain black-alkali soils were saturated with monovalent base. Obviously this method does not enable us to say whether the replaceable divalent base is calcium or magnesium or both. We have used this method in the present study.

The procedure adopted in this study was as follows: After digesting 25 grams of soil over night at 70° C. with 250 cc. normal ammonium chlorid, the sample was thrown on a filter and leached to one liter with normal ammonium chlorid. The leachate was then analyzed for bases. The soil remaining on the filter was freed from ammonium chlorid by leaching with methyl alcohol as proposed by Kelley and Brown,[15] [7] and then the absorbed NH_4 was determined by distillation in the presence of an alkali. The amount of absorbed NH_4 is assumed to be equivalent to the sum of all the bases that have been replaced. Leach-

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[6] It is doubtful whether the barium chlorid method as proposed by Burgess and Breazeale[5] fulfills this requirement.

[7] This method was discussed at the Meeting of the International Society of Soil Science held in Washington, D. C., June, 1927.

ing with methyl alcohol makes it possible to remove the occluded ammonium chlorid without danger of loss of the absorbed NH_4 through hydrolysis, and the coagulating effect of the alcohol prevents the leaching out of colloidal materials. When water is used to leach out the ammonium chlorid, more or less colloidal material may pass through the filter paper. With soils which contain considerable organic matter it may be necessary to make a correction in the NH_4 data by distilling the original soil, but this is not important with the Fresno soil.

TABLE 9

EFFECT OF GYPSUM ON THE REPLACEABLE BASES

(Milliequivalents per 100 grams.)

	Plot 4									
Depth in inches	Before treatment					After treatment				
	K	Na	Ca+Mg	Total (S)	Na as per cent of total	K	Na	Ca+Mg	Total (S)	Na as per cent of total
0–12	0	3 70	1.10	4.80	75	0	1.78	3.17	4.95	36
12–24	0	3.42	1.52	4.94	69	0	2.00	2.95	4.95	40
24–36	0	2 39	2.53	4.92	49	0	2.57	2.32	4.89	53
36–48	0	2.01	3.22	5.23	38	0	3.13	2.28	5.41	58
	Plot 11									
0–12	0.49	3.44	1.12	5.05	68	0.11	1.97	2.92	5.00	39
12–24	0.05	3.24	2 10	5.39	60	0	2.33	2.96	5.29	44
24–36	0	1.71	4.38	6 09	28	0	2 33	3.73	6 06	38
36–48	0	1.18	5.18	6.36	19	0	1.49	4.87	6.36	23

TABLE 10

EFFECT OF SULFUR ON THE REPLACEABLE BASES

(Milliequivalents per 100 grams.)

	Plot 10									
Depth in inches	Before treatment					After treatment				
	K	Na	Ca+Mg	Total (S)	Na as per cent of total	K	Na	Ca+Mg	Total (S)	Na as per cent of total
0–12	0.26	2 69	2.60	5.57	48	0.08	2.02	3.65	5.75	35
12–24	0.12	3.07	2.40	5.59	55	0.05	1.35	4.15	5.55	24
24–36	0.04	2.81	3.20	6.05	46	0 02	1.57	4.62	6.21	25
36–48	0.07	2.50	3.38	5.95	42	0.05	1.73	4.35	6.13	28
	Plot 19									
0–12	0.36	3.31	2.07	5.74	58	0.15	2.39	3.42	5.96	40
12–24	0.09	2.85	2.93	5.87	49	0.04	2.10	3.41	5.55	38
24–36	0.06	2.72	3.39	6.19	44	0	2.05	3.90	5.95	34
36–48	0.10	2.82	3.78	6.70	42	0	2.18	4.48	6.66	33

This method rests on the assumption that the soil contains no compounds of sodium or potassium, other than the water-soluble salts, that are soluble in ammonium chlorid solution. The fact that practically no potassium was found in the samples drawn after the materials had been applied is strong evidence in support of this assumption. It should be borne in mind, however, that when we are dealing with a soil which has a low total replaceable-base content, such as the Fresno soil, small amounts of impurities in the chemical reagents, together with only slight solubility effects, may materially affect the relative percentage of sodium found.

TABLE 11

EFFECT OF IRON SULFATE ON THE REPLACEABLE BASES

(Milliequivalents per 100 grams.)

Plot 17

Depth in inches	Before treatment					After treatment				
	K	Na	Ca+Mg	Total (S)	Na as per cent of total	K	Na	Ca+Mg	Total (S)	Na as per cent of total
0–12	0.46	3.14	1 38	4.98	63	0 09	2.34	2 74	5 17	45
12–24	0.21	2 64	2.35	5 20	51	0 04	2.41	2.83	5 28	46
24–36	0.10	2 12	2.99	5 21	41	0.02	2 61	2 57	5 20	50
36–48	0	2.29	2 81	5.10	45	0 03	2.34	2.70	5.07	46

Plot 45

0–12	0.30	3 09	2.54	5.93	52	0	1.53	4.48	6 01	25
12–24	0.12	2.84	3.10	6.06	47	0	2 00	4 01	6.01	33
24 36	0 00 .	2.05	4.65	6 70	31	0	1.85	4 91	6 76	27
36–48	0.03	2 16	4.34	6.53	33	0	1.22	5.50	6.72	18

As shown in tables 9–12 the content of replaceable monovalent base (chiefly sodium) has been decreased and the content of replaceable divalent base has been materially increased by each of the treatments that have been applied. This effect has been most marked in the upper layers of the soil. With certain of the plot this effect has been confined entirely to the first and second feet. The relationship of replaceable sodium to total replaceable base is especially well brought out by expressing the sodium as per cent of the total content of replaceable bases. In his previous work on Hungarian alkali soils Arany[1] brought out a similar relationship by showing the effects produced by certain treatments on the so-called alkali quotient of the soil, which quotient was determined by dividing the sum of the

replaceable sodium and potassium by the total replaceable bases. Since solubility effects and impurities in the reagents may have been involved in the sodium determinations, these data should not be considered as absolute. However, the differences found in the samples taken before and after the treatments were applied are fairly consistent. The relationship of the replaceable monovalent bases (sodium in particular) to the total replaceable bases, is a fundamentally important aspect of alkali soils, as has been emphasized in previous publications from this and other laboratories.

TABLE 12

EFFECT OF ALUM ON THE REPLACEABLE BASES

(Milliequivalents per 100 grams.)

Plot 18

Depth in inches	Before treatment					After treatment				
	K	Na	Ca+Mg	Total (S)	Na as per cent of total	K	Na	Ca+Mg	Total (S)	Na as per cent of total
0-12	0 40	3 68	0 87	4 95	74	0.09	1.72	3.04	4.85	35
12-24	0.00	2 90	1 82	4 72	61	0 04	1.37	3.22	4.63	30
24-36	0 00	3 61	1 84	5 45	66	0.04	1 89	3 22	5 15	37
36-48	0.00	3 47	1 86	5 33	65	0 00	1 84	3 19	5 03	37

CALCIUM AND MAGNESIUM EXTRACTED WITH NH_4Cl

The solutions obtained by leaching the samples of soil with normal ammonium chlorid were analyzed for calcium and magnesium as well as for potassium and sodium. The assumption was made that the loss in water-insoluble carbonates incident to leaching with ammonium chlorid was due to the solution of normal carbonate of calcium and magnesium. If this assumption is correct, the ammonium chlorid extracts must have contained calcium or magnesium, or both, derived from carbonate forms, in amounts equivalent to the loss in CO_3. The amounts[8] thus calculated are recorded in tables 13 to 19, column 4. The replaceable calcium and magnesium, calculated by the method already discussed [NH_4 absorbed — replaceable $(K + Na)$] is given in column 5. The sum of the replaceable and the dissolved-carbonate forms substracted from the total amounts of these bases

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[8] If the soil contains basic carbonate of magnesium, the data calculated on the basis of normal carbonate are probably too low.

found must represent still other forms of calcium and magnesium, probably silicates. Data obtained by this method of calculation are reported in column 6 of these tables.

TABLE 13

Plot 4, Effect of Gypsum on the Calcium and Magnesium Constituents; Amounts Extracted with NH₄Cl Solution

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg ([HN₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment .	5 42	2 02	7 44	1 56	1 10	4 78
1st ft. after treatment	7 87	1 79	9 66	1 60	3 17	4 89
2nd ft. before treatment .	4 57	1 71	6 28	0 31	1 52	4 45
2nd ft. after treatment	6 01	1 68	7 69	0 21	2 95	4 53
3rd ft. before treatment . . .	4 77	2 25	7 02	2 31	2 53	2 18
3rd ft. after treatment .	4 96	1 91	6 87	2 33	2 32	2 22
4th ft. before treatment .	5.42	2 49	7 91	3 12	3 22	1 57
4th ft. after treatment.. . .	4 30	1 84	6 14	2 80	2 28	1 06

TABLE 14

Plot 10, Effect of Sulfur on the Calcium and Magnesium Constituents; Amounts Extracted with NH₄Cl Solution

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg [NH₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment.... .	9 05	2 82	11 87	0 79	2 60	8 48
1st ft. after treatment ..	8 64	2.37	11 01	0 50	3.65	6 86
2nd ft. before treatment	6 29	2.49	8 78	0 22	2 40	6 16
2nd ft. after treatment	8.71	2 55	11 26	0 93	4 15	6 18
3rd ft. before treatment . ..	14.43	4 35	18 78	12 99	3 20	2.59
3rd ft. after treatment.....	26 01	4 38	30.39	21 73	4 62	4 04
4th ft. before treatment ..	20 41	4 71	25 12	21 81	3.38	−0.07
4th ft. after treatment.....	29 16	4 74	33 90	27.02	4 35	2 53

It will be noted that in each instance a greater amount of calcium than magnesium was extracted and that with the exception of plot 4 the quantities of calcium found and of carbonate dissolved were much

greater in the third and fourth feet than in the first and second feet. On the other hand there was not a corresponding consistent relationship as regards magnesium. The data indicate that calcium carbonate is the predominant but not the only water-insoluble carbonate in this soil.

TABLE 15

PLOT 11, EFFECT OF GYPSUM ON THE CALCIUM AND MAGNESIUM CONSTITUENTS;
AMOUNTS EXTRACTED WITH NH_4Cl SOLUTION

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg [NH₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment......	7 89	2 43	10 32	6 94	1 12	2.26
1st ft. after treatment . . .	8 66	2 40	11 06	6 60	2.92	1.54
2nd ft. before treatment	5 60	2.36	7.96	5.06	2.10	0.80
2nd ft. after treatment	8 42	2.29	10.71	4.80	2.96	2.95
3rd ft. before treatment . . .	20.03	4.69	24.72	19.87	4 38	0 47
3rd ft. after treatment... ...	16 08	3.64	19.72	13.33	3.73	2.66
4th ft. before treatment...	29 92	5 68	35.60	23 56	5 18	6 86
4th ft. after treatment......	27.32	5.23	32 55	23 11	4 87	4 57

TABLE 16

PLOT 17, EFFECT OF IRON SULFATE ON THE CALCIUM AND MAGNESIUM
CONSTITUENTS; AMOUNTS EXTRACTED WITH NH_4Cl SOLUTION

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg [NH₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment...........	9.70	4 20	13 90	1.91	1.38	10.61
1st ft. after treatment	9.15	2.51	11.66	2.09	2.74	6.83
2nd ft. before treatment... ..	8 09	4.74	12 83	1.04	2.35	9.44
2nd ft. after treatment	9.43	3.56	12 99	2.47	2.83	7.69
3rd ft. before treatment	22.62	5.06	27.68	18.55	2 99	6.14
3rd ft. after treatment	30.30	4.85	35.15	29.49	2.57	3.09
4th ft. before treatment	34.75	5.71	40.46	29.16	2.81	8.49
4th ft. after treatment...........	28 45	4.48	32.93	24.31	2.70	5.92

The calculations reported in column 6 of the tables support the view that this soil contains silicates of calcium or magnesium or both that are soluble in ammonium chlorid solution. The different plots and the various depths of a given plot differ in this regard. The upper horizon of plot 11 seems to contain considerably less soluble silicate than that of the other plots.

TABLE 17

PLOT 18, EFFECT OF ALUM ON THE CALCIUM AND MAGNESIUM CONSTITUENTS; AMOUNTS EXTRACTED WITH NH₄Cl SOLUTION

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg [NH₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment...... .. .	8.86	4 23	13 09	5.15	0 87	7.07
1st ft. after treatment	9.57	2 98	12 55	3 41	3 04	6 10
2nd ft. before treatment ...	10 15	4 99	15 14	6 41	1 82	6 91
2nd ft. after treatment ...	13 57	4 24	17 81	6 27	3 22	8 32
3rd ft. before treatment	38 63	8 35	46 98	40 72	1 84	4 42
3rd ft. after treatment...	37.02	7 61	44 63	41 52	3 22	−0 11
4th ft. before treatment	41 53	7 93	49.46	46 09	1 86	1 51
4th ft. after treatment	30.87	5 18	36 05	26 82	3 19	6 04

As regards the effects of the treatments that have been applied to these plots, the data show that the extractable magnesium has been slightly decreased and the total replaceable divalent base has been substantially increased. With the exception of plot 4, which was treated with 15 tons per acre of gypsum, the data reported in column 6 of tables 13 to 19 indicate that the soluble silicate of the first foot has also been decreased. Although the methods of determination, upon the results of which these calculations are based, are not highly accurate, especially where relatively large amounts of carbonates occur, it seems probable that this black-alkali soil contains a small amount of some easily decomposable calcium silicate which is acted upon by the products of sulfur oxidation and acidic substances like iron sulfate and alum. The calcium of such compounds, if present, would thus be caused to play an important part in the reclamation process. The black-alkali soil near Salt Lake City, Utah, appears to contain relatively large amounts of easily decomposable silicate of calcium.[13]

TABLE 18

PLOT 19, EFFECT OF SULFUR ON THE CALCIUM AND MAGNESIUM CONSTITUENTS;
AMOUNTS EXTRACTED WITH NH₄Cl SOLUTION

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg [NH₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment....	9.89	3 80	13 69	2.91	2.08	8.70
1st ft. after treatment	8.43	3.58	12 01	1.75	3 42	6.84
2nd ft. before treatment.... ..	7 80	4 16	11 96	1.16	2.93	7.87
2nd ft. after treatment ..	7.15	3.74	10 89	1.94	3.45	5.50
3rd ft. before treatment . ..	20 52	5 02	25.54	13 65	3.39	8.50
3rd ft. after treatment..... .	22.30	5.03	27 33	15 85	3.90	7.58
4th ft. before treatment	35.32	7 25	42.57	28 85	3.78	9.94
4th ft. after treatment.	32 16	6.24	38.40	29.77	4.48	4.15

TABLE 19

PLOT 45, EFFECT OF IRON SULFATE ON THE CALCIUM AND MAGNESIUM
CONSTITUENTS; AMOUNTS EXTRACTED WITH NH₄Cl SOLUTION

(Milliequivalents per 100 grams.)

	1	2	3	4	5	6
	Ca	Mg	Total Ca+Mg	Ca+Mg as carbonates	Replaced Ca+Mg [NH₄−(K +Na)]	Ca+Mg as compounds other than replaceable and carbonate forms (3-4-5)
1st ft. before treatment	6.15	4.41	10.56	0.89	2.54	7.13
1st ft. after treatment..	8 29	3.91	12.20	0.86	4.48	6.86
2nd ft. before treatment..... .	5.30	4.56	9.86	1.88	3.10	4.88
2nd ft. after treatment....	7.35	4.22	11.57	1.96	4.01	5.60
3rd ft. before treatment..	15.86	5.31	21.17	9.59	4.65	6.93
3rd ft. after treatment........... .	15.58	5.27	20.85	10.86	4.91	5.08
4th ft. before treatment........	16.86	4.89	21.75	14.9	4.34	2.45
4th ft. after treatment..............	26.95	5.85	32.80	23.02	5.50	4.28

GENERAL DISCUSSION

The preceding data show that under the influence of the treatments that have been applied, this soil is being transformed into a normal soil, although the transformation has not yet become complete. Now that the several treatments have brought about a substantial reduction in the content of soluble carbonate, the replacement of a considerable part of the replaceable sodium by divalent base (probably calcium) and a material reduction in the OII-ion concentration (table 20), careful management of the soil as regards the drainage conditions and irrigation practice should promote a continuation of these changes. Under these conditions the products of biological agents, acting on the calcium carbonate of the soil, will gradually bring about a further replacement of sodium by calcium, and good drainage conditions will make it possible to leach away the soluble sodium salts that are formed.

TABLE 20

EFFECT ON THE pH VALUE OF THE SOIL

Depth in inches	Plot 4		Plot 11		Plot 10		Plot 19	
	Before treatment	After treatment with gypsum	Before treatment	After treatment with gypsum	Before treatment	After treatment with sulfur	Before treatment	After treatment with sulfur
0-12	9.55	8 20	9 72	8 54	9 70	7 49	10 10	9 15
12-24	9 72	8 94	9 72	9.67	9.70	8 63	9.86	9.51
24-36	9.69	9 26	9 55	9 37	9 82	9 49	9.71	9 10
36-48	9.38	9 26	9.52	9 30	9.38	9 65	9 37	8 73

Depth in inches	Plot 17		Plot 45		Plot 18	
	Before treatment	After treatment with iron sulfate	Before treatment	After treatment with iron sulfate	Before treatment	After treatment with alum
0-12	9.77	8.85	9.70	8.88	9 86	8 78
12-24	9.52	9 25	9.62	9.54	9 70	9 19
24-36	9.37	9.17	9.44	9 37	9 37	9 04
36-48	9.04	8.85	8.86	8.73	9 35	8.79

As stated already the application of gypsum brings about two important chemical reactions in black-alkali soils; namely, the conversion of (*a*) sodium carbonate into sodium sulfate and calcium carbonate, and of (*b*) sodium-exchange compounds into calcium compounds and sodium sulfate. Neither of these reactions can go to

completion unless the soluble sodium salts are leached out. Moreover, these reactions take place simultaneously. Hence, the amount of gypsum required is dependent upon the content of both sodium carbonate and replaceable sodium, and, as suggested above, it may be necessary to leach the soil to remove the soluble products. It is interesting to note in this connection that Loughridge[19] pointed out as early as 1897 that from two to three times as much gypsum should be applied as is indicated by the soluble carbonate content of the soil. The reason for this fact was not known at that time.

The important reactions which take place when sulfur undergoes oxidation in alkali soils were recently discussed by Samuels.[20] Just as is the case with gypsum, the sulfur-oxidation product (sulfuric acid) reacts with soluble carbonate and sodium-exchange compounds simultaneously. Calcium carbonate, if present, is also acted upon, and probably certain calcium silicates as well. As shown by Samuels, the intermediate reactions are complex. They ultimately lead, however, to the neutralization of the alkaline conditions of the soil by converting the soluble carbonate into sulfate and bicarbonate and the sodium-exchange complex into calcium compounds through the effect produced on calcium carbonate and silicate. The soluble salts can then be leached out.

That sulfur brings about an activation of the water-insoluble calcium compounds is possibly the most fundamentally important factor connected with its action on an alkali soil. This phase of the question has been discussed in previous publications from this laboratory, [13, 17, 20] but its importance justifies further emphasis. Fortunately, American alkali soils usually contain an abundance of calcium carbonate. This, however, is not true of certain important alkali areas of Europe. With the latter it is not probable that the application of sulfur will be satisfactory unless lime is also applied.

It is probable that the chemical changes that have taken place in plot 19, as regards both carbonates and replaceable bases, were produced by the joint action of the manure that was applied in 1922 and the sulfur applied in 1925, but the proportion of the changes that was produced by each of these materials can not now be determined. It seems that the major part of the reactions was caused by the sulfur, since analyses, not reported herein, failed to reveal any pronounced change in the water-soluble carbonate of this plot until several months after the sulfur was applied. Moreover, barley, sown for two successive years after the manure had been applied but before sulfur was applied, failed to germinate over most of this plot.

However, it is not until the soluble carbonate has been largely removed that crops like barley and alfalfa will grow on this soil and the decomposition of soluble carbonate goes hand in hand with reactions involving the exchange complex.

The decomposition products of the manure probably initiated the necessary chemical reactions and the oxidation product of the sulfur carried them further towards completion. The manure underwent rapid decomposition indicating active micro-biological action.[9] It is also of interest that the rate of sulfur oxidation on this plot, as shown by evidence of sulfate formation and the germination and growth of alfalfa sown only three months after the sulfur was applied, has been especially active. Probably the micro-organisms contained in the manure and the chemical and physical effects produced by its decomposition were conducive to the rapid oxidation of sulfur.

An understanding of the chemical effects of iron sulfate and alum necessitates giving first consideration to hydrolysis. These compounds are acidic by virtue of the fact that they undergo hydrolysis, one of the hydrolytic products of each being sulfuric acid. It is the hydrogen ion thus formed that is responsible for the marked effect of these materials. It is well known, of course, that carbonates are decomposed by soluble aluminum salts with the evolution of CO_2 and the precipitation of aluminum hydroxid. As to the effect of aluminum salts upon the base-exchange constituents, Kelley and Brown[14] showed that when a dilute solution of aluminum chlorid is added, the replacement of soil bases is brought about by the hydrogen ion formed by hydrolysis and not by the aluminum ion. In addition to these effects it is well known that aluminum and iron salts produce marked flocculation of soil colloids.

It follows from the preceding that the application of alum will bring about a decomposition of carbonates and the transformation of the sodium-exchange complex into calcium compounds, very much as is the case where sulfur is applied. The determination of the water-soluble constituents reported in table 5 and the replaceable bases shown in table 12 fully confirms this view. The aluminum hydroxid formed by hydrolysis was probably precipitated colloidally by the electro-negative soil colloids. Later, when the soil dried out, the aluminum hydroxid probably passed into aluminum oxid by dehydration and thus became a relatively stable and inert component of the soil.

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[9] Since micro-organisms are known to be more or less active in many black-alkali soils, carbon dioxid must be formed therein, notwithstanding the claim (3, 4) that carbon dioxid is non-existent in this kind of soil.

While iron sulfate probably brings about a partial precipitation of the soluble carbonate as iron carbonate, the fundamentally important chemical reactions produced by it are largely those involving the action of the hydrogen ion. In this case also, as shown in tables 4, 7, 8 and 11, the hydrogen ion decomposed soluble carbonates and converted sodium-exchange complex into calcium compounds through the intermediate agency of calcium carbonate. The ferrous hydroxid formed by hydrolysis was absorbed by the soil colloids and was later oxidized into the ferric form and thus became essentially inert.

Thus it will be seen that there is a fundamental similarity in the reactions produced by oxidizing sulfur, alum and iron sulfate. The rates at which these substances react, however, are widely different. Sulfur, being dependent upon biological agents for its oxidation, must necessarily react relatively slowly. Iron sulfate and alum, on the other hand, being highly soluble substances, react at once. It is probable that the full chemical effect of these last named substances is exerted as soon as they are brought into contact with the soil particles through the solvent and leaching action of water.

Soon after alum and iron sulfate were applied to these plots pronounced effects were noted in the chemical and physical properties of the soil. Although these materials were applied more than five years ago (1922), and the soil has been leached and irrigated freely since that time, there is as yet no indication of a reversal of the beneficial chemical and physical effects. The plots, although originally highly puddled and impervious, have continued to absorb water freely since these materials were applied, their tilth is good and on each of them the growth of alfalfa is excellent. The beneficial physical effects are probably due chiefly to the chemical reactions referred to above and not to the coagulation of the colloids of the soil independent of the chemical changes. When this is the case leaching will, therefore, probably not bring about a reversal of the effects, as Joffe and McLean[10] inferred would be the case. In fact it appears to be impossible to coagulate the colloids of this soil by means of aluminum or iron salts without decomposing carbonates and altering the chemical nature of the exchange complex, and these chemical reactions are the important effects which aluminum and iron salts produce.

As stated already, each of the materials that have been applied has reacted with the carbonates and the base-exchange complex of the soil. It does not follow, however, that the amount of chemical change which these constituents have undergone, is a reliable measure of the chemical efficiency of the applied materials. In addition to the effects

produced by these materials, the calcium content of the irrigation water and carbon dioxid formed by micro-biological agents and the growth of crops have taken part in the chemical reactions. Moreover, there has been some calcium and sulfate lost by leaching and absorbed by the crops. Finally the fact that this soil is extremely variable, as was shown previously,[11] should also be borne in mind. Since the magnitude of these various factors is unknown, it is not possible to determine the precise chemical efficiency of the applied materials. The data, however, clearly establish the qualitative nature of the reactions.

From a practical standpoint, the data also suggest that it is not necessary to apply any of these materials in amounts equivalent to the total soluble-carbonate and replaceable-sodium content of the soil. When materials of these kinds are applied the reactions will bring about a reduction in the OH-ion concentration in consequence of which micro-biological action will be stimulated. This latter will lead to the formation of carbon dioxid and the gradual solution of calcium minerals in the soil, which in turn will react with the base-exchange complex and ultimately serve to stimulate plant growth. When the chemical reactions produced by these various means have reached the point where a crop like alfalfa can be grown satisfactorily, it seems probable that the remaining portion of the needed chemical change can be effected through proper management of the soil. By plowing down green manures or farm manures and the liberal use of irrigation water, the calcium minerals of the soil will gradually react with the exchange complex and the replaced sodium will be leached out. Thus the soil constituents will ultimately become essentially normal in .composition.

As was pointed out above the growth of crops has been markedly stimulated by the application of these materials. The most pronounced effect has been in those cases where the chemical changes in the soluble carbonates and the base-exchange constituents have been the greatest. As will be seen by reference to the crop records reported in a separate paper,[18] there is good agreement between the effects on crops and the chemical changes referred to above. On the other hand, the untreated check plots of these experiments have not produced satisfactory yields. In fact barley and alfalfa still fail to grow satisfactorily on the greater part of the check plots, whereas large yields of alfalfa are being obtained from the plots that have been discussed in this paper.

SUMMARY

The application of gypsum, sulfur, iron sulfate and alum has produced important chemical changes in the black-alkali soil near Fresno, California. With each of these materials the chemical reactions have involved the soluble carbonate and the exchange complex of the soil. It was found that gypsum has precipitated the soluble carbonate as calcium carbonate in accordance with theory, while the other materials have either decomposed carbonate or else converted it into bicarbonate. Simultaneously with the effect on soluble carbonate, the exchange complex has been acted upon.

The effect of gypsum is dependent on its soluble calcium, while sulfur, iron sulfate and alum are effective because of the H ions that are formed. The acid formed by the oxidation of sulfur, or the hydrolysis of iron sulfate and alum, dissolves calcium carbonate, and possibly other minerals, and thus brings calcium into solution.. As a result the sodium content of the exchange complex is decreased and the calcium content is increased. In consequence of these chemical transformations the physical conditions of the soil have been markedly improved and the growth of crops has been pronouncedly stimulated.

The theoretical and practical aspects of the black-alkali soil problem have been discussed.

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CHANGES IN COMPOSITION DURING RIPENING AND STORAGE .OF MELONS

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The shipment of various kinds of melons from the western states to eastern cities has become an important item in the produce industry during recent years. Because of the distance from producing regions to the larger markets, most of these melons are harvested and shipped in a more or less immature condition. Practices in harvesting and shipping should be established to yield the most satisfactory results from the standpoint of both shipper and consumer. Aside from some investigations with cantaloupes, little work has yet been done to establish rational methods for harvesting and handling of melons. Hence a detailed study has been undertaken at the University Farm, Davis, of the changes in composition and in quality of the leading types of melons, both in fruit ripening naturally on the plant, and in fruit harvested more or less immature and stored under different conditions.

Of the factors influencing quality of melons from the consumer's point of view, sweetness is probably most important. This may be affected both by the amount of. sugars present, and by the kinds of sugars. The texture is also important, for palatability is partly determined by the degree of softness and the juciness of the flesh. From the shipper's standpoint, firmness is most important, for it determines the physical limits of shipping range and market handling.

CHANGES DURING RIPENING ON THE PLANT

The various classes of sugars, which chiefly affect flavor and sweetness, were determined in fruit at different stages of growth. Determinations of the pectic substances in the fruit-flesh at successive stages of development indicated the changes in composition connected with softening of the fruit. Strains which had been inbred for one or more years were used, to obtain greater uniformity of material.

Selection of Sample Material.—For the work with cantaloupes, fruit of the Salmon Tint variety, grown at Davis, was used. Flowers were tagged on June 28, 1926. Under midsummer conditions at Davis, this variety attains the full-ripe condition in 40 to 45 days after the fruit is set. The tagged fruit furnished material of uniform age for the chemical and storage experiments. Beginning 25 days after the fruit set, samples were picked every three days, until the full or field-ripe stage was reached.

For studies on the Honey Dew melons, flowers were tagged July 20, 1927. This variety requires about 55 days to reach commercial maturity, at which stage the fruits are still firm but the flesh is edible. For about 10 days following this, the ripening process continues to the full ripe condition, then deterioration commences if the fruit is left in the field. Samples were taken for analyses at intervals during the last three weeks of the ripening period.

Flowers of the Golden Beauty Casaba were tagged on July 24, 1927. This variety attains commercial maturity in about the same time as the Honey Dew, but its later stages of ripening proceed more slowly. Samples were taken of Casaba during the last five weeks of the ripening period.

Preparation of Samples.—Six melons of the same age, of about the same degree of maturity externally, but of somewhat varying sizes, were used for each sample. The number of fruits is considered sufficient to give reliable results in the measurement of the rather large differences between successive samples which were involved in most of this work.

A longitudinal segment was taken from each fruit, the seeds and placenta were removed, and a layer about one-eighth of an inch thick (including the rind) was pared from the outside and discarded. The flesh was ground in a food chopper, mixed well, and 300-gram samples were weighed out for sugar analysis. The samples were placed in

large flasks at once, covered with 600 cc of hot 95 per cent alcohol, and boiled for 2 or 3 minutes. Calcium carbonate was added to the sugar samples. The samples were stored about 3 months before extraction.

Methods of Analysis.—Duplicate samples of the pulp were weighed out for determination of total solids. These were evaporated on the waterbath, then dried in the oven at 80° C. Three weeks or longer were required for the dried pulp to reach approximately constant weight. It is realized that there may be some loss of solids during this prolonged drying, so that the "total solids" reported may be a little low.

The juice was expressed from the remainder of the pulp for determination of specific gravity with the Brix spindle.

The sugars were completely extracted from the stored sample with 55 per cent alcohol. Starch and other hydrolyzable polysaccharides were determined on the residue, while the extract was used, after clarification with basic lead acetate, for reducing sugars and sucrose determination. The sucrose was inverted by digestion for about two hours with 1 cc of technical invertase (Wallerstein Laboratories, New York) to 100 cc of sugar solution. Sugars were determined by the combined Munson-Walker and Schaffer-Hartman methods. Reducing and total sugars are calculated as "invert sugars," and the difference between them $\times$ .95 is taken to be sucrose. Levulose was determined on the alcohol extract by the Nyn's method. The polysaccharides given in the last column of table 1 were determined after hydrolysis of the alcohol-insoluble residue with 5 per cent HCL.

Sugars in Cantaloupes.—An extensive study of the sugar content as determined on the juice of immature, half-slip, full-slip and field ripe cantaloupes has been reported by Chase, Church and Denny.[6] The differences reported by these workers between the foregoing classes of melons were not as great as when the melons were classed according to edibility. In general their data show more total sugars in high quality melons than in those of low quality. The soluble solids, as determined by the Brix spindle, were higher in the high quality melons. As a result of the latter work, Chase, Church and Denny were able to conclude that melons whose juice gave a Brix reading of 10 or above were likely to be of good quality, those between 9 and 10 were of doubtful quality, and those below 9 were of poor quality.

Table 1 gives the results obtained with the 1926 series of cantaloupes at Davis. The results here were obtained by analysis of the whole flesh, as described, rather than of the juice alone, as was done

by Chase, Church and Denny. Marked correlation between the changes in the different constituents and the age of the fruit, is to be observed.

TABLE 1

Changes in Composition of Cantaloupe Melons During Growth and Ripening, in Per Cent of Fresh Weight of Flesh. Harvested

July 23 to August 10, 1926

Number of days after fruit-setting	Conditions of fruit	Total solids	Brix reading on juice	Reducing sugars	Levu- lose	Sucrose	Total sugar	Hydro- lysable polysac- charides
25	Inedible, flesh white.........	6.00	5.9	3.01	1.56	0.17	3.29	0.53
28	Inedible, flesh faint pink..	7.25	7.1	3.40	1.84	0.83	4.27	0.51
31	Hard but distinctly sweet, flesh medium pink.........	9.70	9.3	3.31	1.77	2.74	6.19	0.50
34	Barely edible, flesh pink	9.82	9.6	3.10	1.77	3.21	6.48	0.48
37	Forced slip, edible, flesh pink................................	10.74	10.8	2.83	1.56	4.37	7.43	0.45
40	Half slip...............................	12.19	11.5	2.43	1.21	5.52	8.24	0.32
43	Full slip..............................	12.16	11.8	2.05	0.94	5.68	8.03	0.28

The cantaloupe fruit increases slightly in average weight and gains steadily in per cent of total solids throughout the latter part of the growth period, the maximum being reached at the half-slip stage. The increase in per cent of solids is primarily due to the increase in sugars and not to loss of water. At the 25-day stage, 52 per cent of the total solids consists of sugar, but at the half-slip stage they constitute 66 per cent of the total. The soluble solids in the juice as shown by the Brix reading show a fairly close agreement with the total sugar content of the flesh.

A marked change takes place in the form in which the sugars occur at different stages of maturity. Up to the time the fruit reaches the "barely edible" stage, at 34 days after setting, the per cent of reducing sugars remains about constant, but thereafter these sugars decrease until the fruit is fully ripe. On the other hand, the per cent of sucrose increases rapidly, from almost zero at the 25-day stage, until the fruit reaches the full-slip condition. Figure 1 shows the changes in composition of cantaloupes, in relation to the age of the fruit. The amount of total solids and total sugars increases in almost a straight-line graph, up to the half-slip stage on August 7, with little increase beyond that stage. The increase of sucrose is more rapid than that of the total sugars; it is largely through fresh accretions of sugar to the fruit during the latter part of its growth period, but

judging from the storage experiments, to be presented later, sucrose also increases during ripening partly at the expense of the reducing sugars.

The changes in proportion of the different sugars are important from the standpoint of flavor. Beister, Wood and Wahlin[2], give the following ratings from the standpoint of sweetness: Sucrose, 100;
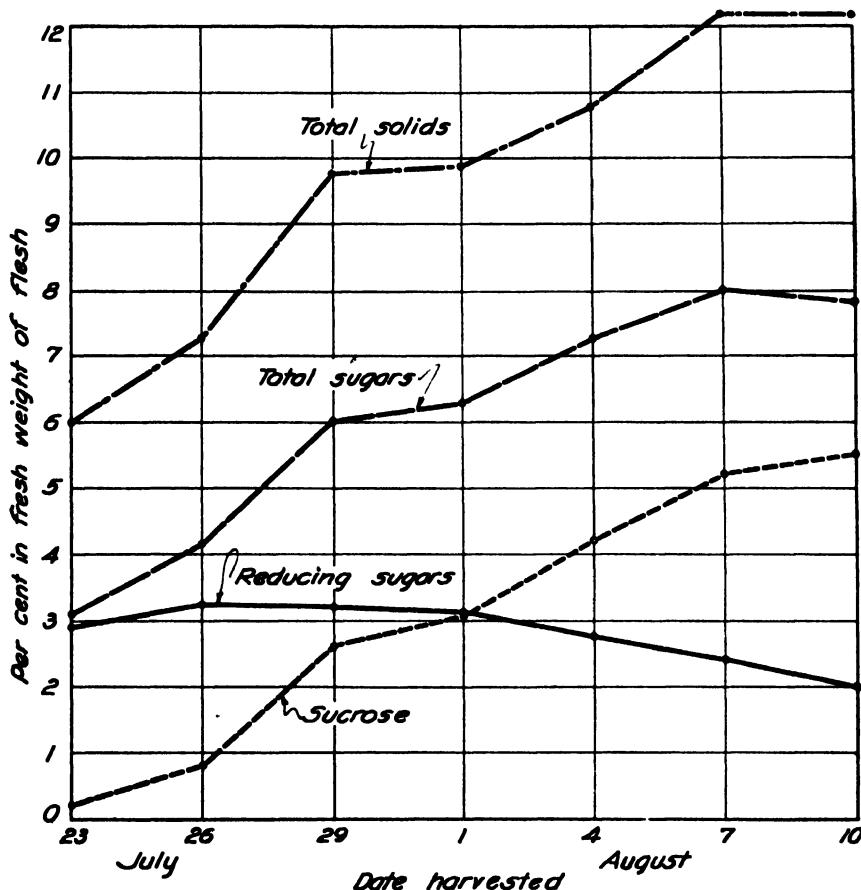

Fig. 1.—Content of sugars and solids in cantaloupe harvested at successive intervals after setting. (Compare with table 1.)

dextrose, 74; levulose, 173, and mixtures of equal parts dextrose and levulose, 130. Table 1 shows that about one-half of the reducing sugars in cantaloupes are levulose, the rest presumably being dextrose. The ratio of levulose to reducing sugars varies only slightly during ripening. Thus, while the fruit loses slightly in sweetness because of

the loss of some levulose, it gains much more through the large increases in sucrose.

It was found that about half of the hydrolyzable polysaccharides consisted of starch (or dextrin) in the immature fruit, but that even this small amount of starch disappeared by the time the fruit approached ripeness. The balance probably consists of pectic substances. It is evident that the cantaloupe contains no extensive reserves of insoluble carbohydrates that might be expected to change to sugar after the fruit is picked.

Sugars in Other Melons.—The first sample of Honey Dew melons was picked September 3, which was 44 days after the fruit had set. While the fruits were approximately full grown at this time, the flesh was hard, and lacked sweetness and flavor. The rind was a glistening greenish white color. When the fruits were 5 days older, the appearance had not changed but the flesh was noticeably sweeter. On September 15, when the fruits were 56 days old, they were in a condition that might be termed "commercially mature." The rinds while still perfectly hard, were showing à slight tinge of yellow, and while the flesh was still too hard to be palatable, it was distinctly sweet and well flavored. Fruit harvested in this stage may be expected to ripen subsequently in storage, with resulting good quality. The last picking, at the age of 63 days, yielded fruit which was slightly soft and distinctly yellow externally, while the flesh was soft and juicy; the fruit was ready for immediate-use.

The first sample of Casabas, picked August 29, or 38 days after the fruit set, was hard, green and inedible. Not until the fourth sampling, on September 15, when the fruit was 54 days old, was it ripe enough for commercial harvesting. At this time the rind was still perfectly hard, but it was turning yellow at the blossom end, whereas the whole surface was overspread with green in the earlier stages. The flesh, while still hard, was of fair flavor, and such fruit was found to be of excellent quality when picked and allowed to ripen in storage. The fruit picked at the two subsequent periods, on September 22 and October 6, showed progressive ripening changes. At the last date, the fruit was of light orange color over nearly the whole surface and was soft at the blossom end, while the flesh was soft, juicy, and ready for immediate use.

The watermelons used for analysis were of the Black Seeded Angeleno variety and the different samples with reference to maturity were selected on the basis of flesh color. The sample designated "ripe," was of fruit about 50 days old. Many individual fruit records

show that watermelons of the Angeleno and Klondyke varieties attain good edible condition in from 45 to 50 days after the fruit sets. The portion of the watermelon fruit used for analysis was the heart flesh, inside of the seed cavities.

Table 2 gives the analyses of the three foregoing varieties of melons, harvested at different ages from time of fruit setting, and therefore in different stages of maturity.

TABLE 2

SOLIDS AND SUGAR CONTENT OF MELONS PICKED FROM THE PLANT AT SUCCESSIVE STAGES OF DEVELOPMENT. EXPRESSED IN PER CENT OF THE FRESH WEIGHT OF FLESH

Variety	Date picked	Quality	Total solids	Sol-uble solids*	Re-ducing sugars	Sucrose	Total sugars
HONEY DEW	Sept. 3	Hard, inedible . .	8 38	78 8	5 48	0 86	6 38
	Sept. 8	Hard, barely edible	10 67		4 38	4 53	9 15
	Sept. 15	Rind hard, flesh firm, edible	11 27	86 8	4 32	5 40	10 00
	Sept. 22	Soft, excellent eating quality	12 53	87 4	3 33	7 39	11 11
CASABA	Aug. 29	Green, hard, inedible	7 12	73 8	4 66	0 41	5 11
	Sept. 3	Rind hard, greenish. Flesh hard, inedible	7 45	74 4	4 58	0 78	5 40
	Sept. 8	Rind hard, greenish. Inner flesh barely edible	8 27	79 7	4 32	1 94	6 36
	Sept. 15	Rind hard, partly yellow. Flesh hard, edible	8 90	82.4	4 10	3 13	7 40
	Sept. 22	Rind hard, mostly yellow. Flesh firm, fairly edible	9 55	82 2	3 97	4 17	8 36
	Oct. 6	Rind hard, light orange. Flesh soft, juicy, sweet	10 81	84.1	2 76	6 24	9 32
WATERMELON	Very im-mature	Flesh yellowish	6 05	90.6	5 30	0 21	5 58
	I m m a-ture .	Flesh pale pink	7 51	93 7	5 60	1 30	6 97
	Ripe	Flesh red, firm	8 65	93 3	3 78	3 91	7.89
	Overripe	Flesh red, mealy ...	8 69	92 4	2 90	4 85	8.00

* As per cent of total solids.

The three kinds of melons discussed in table 2 show similar changes during maturation while the fruit remains attached to the plant. The total solids (per cent of dry matter) increases steadily, throughout the period under consideration. However, the proportion of these solids occurring in soluble form also increases, which is one reason for the apparent increase in juiciness as the fruit ripens. Soluble solids are lowest at all stages in the Casaba, and are highest in the watermelons. The soluble solids given in table 2 represent that portion of the fruit's solids which were dissolved in 55 per cent alcohol and consist mainly of the various sugars.

The reducing sugars, about constant in amount during the late growth phase, decrease rapidly as ripening commences, in these three types of melons. Levulose was determined only on the Honey Dew melons and is not included in the table. It constitutes about one-half of the reducing sugars throughout the whole series, as was the case in
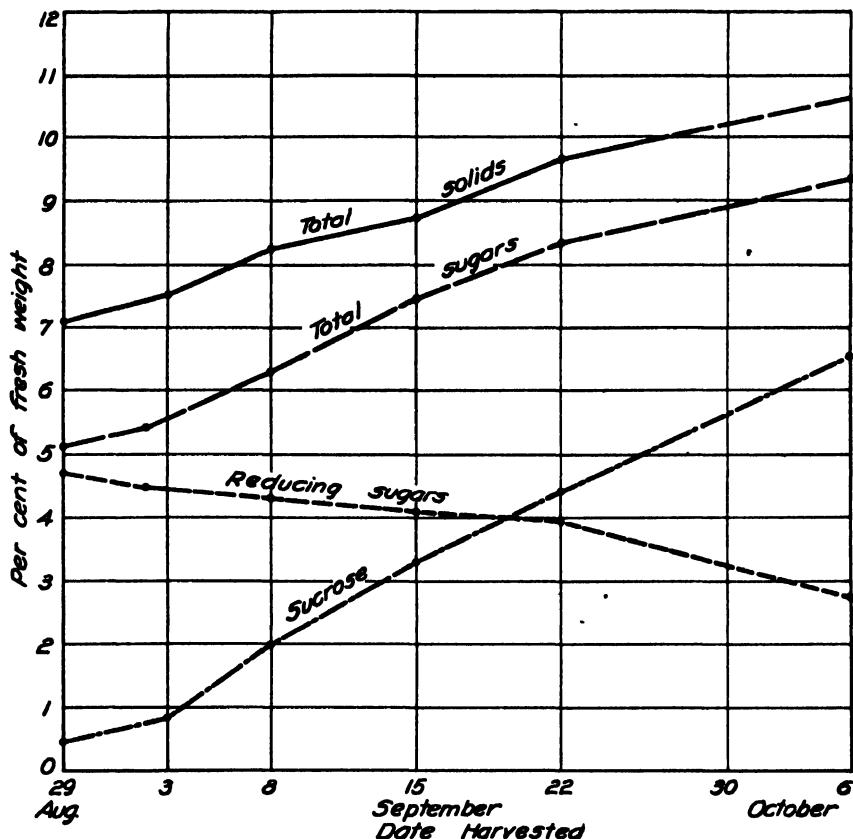

Fig. 2.—Changes in the amount of sugars and solids in Casaba melons, in fruit picked at different ages. (Compare with table 2.)

cantaloupes. Sucrose increases rapidly, from practically none in immature fruit, until it constitutes well over two-thirds of the total sugar content in ripe fruit. The total sugar also increases, although at a more moderate rate, throughout the late-growth and maturation phases of the fruit. Maximum sugar content is not reached until the fruit is in the stage fit for immediate consumption. Fig. 2 shows the changes in sugar content, in relation to age of the fruit, in the Casaba melon.

Pectic Substances.—Investigations on various fruits have indicated that there are three main classes of pectic substances. They are associated with, or are component parts of, the cell walls, and are therefore of special interest in changes involving the softening of fruit. Three classes of pectic substances are considered to be as follows: (1) Pectin, a water-soluble substance which develops during ripening. (2) Protopectin (pectose), an insoluble cell-wall substance which is thought to be changed to pectin during the ripening process. (3) a complex containing pectic acid or a salt of pectic acid, which partially or entirely constitutes the middle lamella, between the cell walls.

By microchemical methods, Carré and Horne[5] demonstrated that in apples the cellulose of the cell wall is intimately associated with protopectin and that the middle lamella is a kind of cement, of a complex containing pectic acid or pectates. They also found numerous globules of a pectic substance accumulating on the walls in the intercellular spaces during the late stages of storage, when the fruit was nearing the final breakdown. These authors concluded that in apples during storage the insoluble protopectin of the cell wall does break down to a soluble form—pectin, which in turn changes to simpler decomposition products. Furthermore, in the later part of the storage period, the pectic acid of the middle lamella is gradually dissolved. They suggest that decomposition of the pectic materials in the cell wall facilitates the outward movement of water, hence the shriveled or wilted condition that often develops in stored fruits.

In melons, which were probably of the cantaloupe type, Mangin states that the cells of the fruit-flesh are held together by short cylindrical connections of pectic substance, which gradually disintegrate, with resultant separation of the cells. Globules of a soluble pectic substance appeared in the intercellular spaces as a result of the disintegration of the cell wall connections.

From the above, it seems that pectic materials are of importance in determining the rigidity of the cell wall, as well as in the cohesion of the cells to each other. The changes which may take place in these materials during ripening and during storage under different conditions, are important in relation to the changes which make the fruit soft, juicy and palatable, and which determine the limits of shipment and storage.

Methods of Determining Pectic Substances.—The pectin, pectic acid and protopectin were determined according to the Carré and Haynes[4] calcium pectate method, with the improvements suggested by Conrad.[7] Samples of 100 grams of freshly ground fruit pulp

were placed in flasks, covered with 300 cc of hot 95 per cent alcohol, and heated to the boiling point. Later, the alcohol was filtered off, and the residue washed with more alcohol to remove most of the remaining sugars. After draining thoroughly, practically all of the alcohol was removed by pressing the residue between filter papers. The residue was then ground with sand (several duplicate determinations at this point showed no difference in results if the sample was oven-dried before the water extraction). Seven or eight successive extractions were made with distilled water, bringing the aqueous extract up to 500 cc. Further extractions usually yielded no trace of soluble pectin.

Either 50 or 100 cc aliquot portions of the water extract were used for the determination of water-soluble pectic acid by precipitation as calcium pectate, the $CaCl_2$ solution being added directly to the water extract for this purpose. Similar aliquots were used for determination of the total soluble pectic substances, after hydrolysis with N/1 NaOH. The difference in yield of calcium pectate before and after hydrolysis is taken to be "pectin," though the calcium pectate values have not been multiplied by any factor to convert them to equivalent pectin values, since the true relationship is not exactly known.

The insoluble residue, after the water extraction, was hydrolyzed with HCl, neutralized, extracted with ammonium citrate, and used for determination of insoluble pectic acid and protopectin, as directed by Conrad. It should be pointed out that pectic acid is partly soluble, at least under certain conditions, hence part of it may be obtained in the water extract, part in the residue. This has been overlooked by previous workers, who have considered only the pectic acid in the residue. Conrad[7] and Appleman and Conrad[1] have questioned the common occurrence of pectic acid or its salts in plant tissues, though they examined only the insoluble residue for its occurrence. However, pectic acid was obtained in many samples in the present work both in the water extracts and in the insoluble portion, especially of the cantaloupe. It is an open question if it occurred as such in the fruit tissues, or was formed during preparation of the samples.

Parallel determinations were made on a large number of samples, using the water extract of freshly ground pulp without killing in hot alcohol, for the determination of soluble pectic substances, as was done by Appleman and Conrad[1] in their work on peaches. The samples handled in this way gave about the same as, or somewhat

higher results than, the duplicates preserved in alcohol, so far as total content of pectic substances is concerned. But the distribution of the pectic constituents by classes was often different by the two methods. Water-extract of fresh pulp gave a higher yield of soluble pectic acid, while the pectin was usually higher in the extract of pulp killed in alcohol. Also the former samples gave a higher proportion of protopectin to pectic acid in the insoluble fractions, than the latter. The use of fresh materials was finally discarded because it was felt that results obtained by this method were open to question on three points: (1) Greater opportunity for enzyme activity to change the form of the pectic constituents in the ground pulp; (2) interference by the presence of other fruit acids that might form insoluble calcium salts; (3) interference by the large amount of sugars present in the water extract of pulp that had not previously been extracted with alcohol. All results reported in this paper were obtained on samples killed and extracted with alcohol.

TABLE 3

PECTIC SUBSTANCES IN CANTALOUPES AT DIFFERENT STAGES OF RIPENING AND AFTER STORAGE. RESULTS IN PER CENT OF FRESH WEIGHT

Condition of the fruit	Water soluble		Insoluble		Total	Per cent soluble
	Pectin	Pectic acid	Pectic acid	Proto-pectin		
Picked before stem slipped.	0.024	0 098	0 054	0 154	0.330	36 9
Picked on half slip	0 056	0 124	0 077	0 085	0 342	52.7
Picked field ripe.	0 059	0 133	0 134	0 010	0 336	57 1
Before slip and 7 days storage at 72° F	0.058	0 118	0 136	0 012	0 320	55.0
Half slip and 7 days storage at 72° F.	0 000	0.183	0 113	0 008	0.304	60.2

Pectic Substances in Cantaloupes.—Fruits of the Salmon Tint variety were gathered in three stages of maturity on the same day, and sampled immediately for analysis. The following classes of fruit were selected: (1) Those that were full grown, but with no evidence of abscission; the flesh was fully colored and sweet, but too hard to be palatable. (2) Fruit in which the abscission from the stem was begun, but in which the rind was still green; the flesh was still firm but more edible than in (1). (3) Fruit that was "field ripe," i.e., the abscission was complete, the rind yellow, and the flesh soft and juicy. Fruits of the two under-ripe classes were stored at ordinary temperature in a cellar for one week, then sampled for analysis.

After storage, they were as yellow and soft as field-ripened melons; the flesh of those picked before stem-abscission, was now soft, juicy and of fair flavor, while that of the half-slip lot was very soft and slightly overripe. The content of the pectic constituents of the above five lots of fruit is given in table 3. The figures in the table are in terms of calcium pectate, as determined for each constituent.

The total amount of pectic materials in the cantaloupe fruit remains about constant during ripening on the plant, with a slight loss during ripening in storage. There is a very marked change in the form of these materials, however. Protopectin, the insoluble cell wall substance, decreases to almost zero during ripening, both in the field and in the cellar-stored fruit. Conversely, there is a marked increase in the water-soluble fraction, both of pectin and pectic acid. There is also a great increase of the pectic acid in the insoluble fraction. These data bear out the theory that the softening of the fruit is associated with the change of the protopectin of the cell wall, to pectin and pectic acid. In the case of the cantaloupe, it appears that pectic acid constitutes the greater portion of the degradation products of protopectin formed during ripening.

Pectic Substances in Other Melons.—Honey Dews, Casabas and watermelons picked in different stages of maturity, were analyzed for their pectic constituents. A description of these samples is given in the earlier part of this bulletin. The water extract of these three kinds of melons never gave more than a trace of water-soluble pectic acid, either on the extract of fresh pulp, or of pulp killed and extracted with alcohol first. Pectic acid and protopectin were determined separately in the insoluble residue and while the actual amount of pectic acid did not always increase with ripening, the proportion of the pectic acid to protopectin generally did increase, though the change was not regular. Therefore it will avoid confusion to consider the pectic constituents of these melons as simply of two classes, the water-soluble pectin, and the water-insoluble pectic substances (pectic acid and protopectin), in the tables.

Table 4 shows that the total pectic content of Honey Dews and Casabas decreases slightly as the fruit becomes riper, thus agreeing with the results obtained by Carré[3] on apples and by Appleman and Conrad[1] on peaches. The total pectic content of watermelons is very much lower than in other kinds of melons, and no well-marked changes in the pectic constituents of the watermelon during ripening are apparent. In the Honey Dew and Casaba varieties, there is a regular and considerable increase in the soluble pectin

content, at the expense of the insoluble fraction, as the fruit becomes riper and its flesh becomes softer and more palatable. This change is shown in the last column of table 4, which gives the per cent of the total pectic constituents which were in water-soluble form. There was about three times as much pectin in the ripe fruit, at the last sampling, as in the hard immature fruit at the first sampling.

TABLE 4

THE PECTIC SUBSTANCES OF MELONS DURING RIPENING ON THE PLANT, EXPRESSED
AS PER CENT OF THE FRESH WEIGHT OF THE FLESH

Sample	Quality	Pectin	Insoluble pectic substances	Total	Per cent soluble
HONEY DEW					
Picked Aug. 29........	Inedible	.055	.253	308	17.9
Picked Sept. 3....... ...	Inedible	.065	.238	.303	21 5
Picked Sept. 8.....	Inedible..	.078	.155	.233	33.5
Picked Sept. 15	Firm, edible	.101	.128	.229	44 1
Picked Sept. 22.. . ..	Soft, edible..	.130	.106	.235	55.3
CASABA					
Picked Aug. 29....	Hard, inedible	.049	.281	.330	14 8
Picked Sept. 3....... . . .	Hard, inedible	.050	.289	.339	14.7
Picked Sept. 8....	Barely edible....	.102	.199	.301	33 9
Picked Sept. 15...	Barely edible... ... . ".	.122	.175	.297	41.1
Picked Sept. 22	Firm, edible	.137	.134	.271	50 6
Picked Oct. 6...........	Soft, juicy	.152	.138	.290	52 4
WATERMELON					
Picked very immature	.. ..	.024	.064	.088	27 3
Picked immature..	.. ..	.012	.072	.084	14 3
Picked ripe	... .	.017	.081	.098	17 3
Picked overripe	.. ..	.017	.070	.087	19.5

CHANGES DURING STORAGE IN FRUIT HARVESTED IMMATURE

From the practical point of view, the nature of the changes occurring in fruit after removal from the plant are of great importance, for they determine the edibility and marketability of the fruit after its long journey from field to consuming center. Knowledge of the probable course of changes within the fruit when it is harvested immature is especially significant, because under present commercial practices most of the melons shipped to market are harvested before full ripeness is attained. It is generally recognized that shipping quality and eating quality are more or less opposed. The work which has been done here is intended to show how far one of these qualities

can be attained without sacrificing the other. Because of the import-
ance of oxidation in ripening processes of fruit, it was considered
advisable to test treatments which prevent access of air to the fruit
flesh. Oiling was one treatment of this kind which was tried. The
fruit which was oiled received a light coat of Frutol (Standard Oil
Co.), before placing in storage. This is a clear, colorless mineral oil.
It did not appear to penetrate into the flesh.

Sugars in Cantaloupes During Storage.—Fruit picked on the half-
slip was stored at 38°F for ten days, then for three days in a cellar
at 72°F. During the cold storage period, there was no softening, the
flesh remaining firm and sweet, but softening occurred during the
subsequent period at higher temperature. The rind, green at first,
yellowed somewhat during cold storage. The oiled fruits were of good
edibility when removed from cold storage, but at the end of three
days at the higher temperature they developed a slightly strong or
sour flavor. Oiled fruits stored at 73°F for a week, without previous
cold storage, developed this undesirable flavor to a marked degree.
It may be ascribed to the accumulation of the products of incomplete
respiration, owing to the reduction in gas exchange by the oil coating.
Table 5 gives the sugar content of the fruit before and after storage
in this experiment.

TABLE 5

SUGAR CONTENT AFTER STORAGE, OF CANTALOUPES PICKED AT HALF SLIP.
EXPRESSED IN PER CENT OF THE FRESH WEIGHT

Treatment	Condition of fruit	Total solids	Brix reading on juice	Re-ducing sugars	Sucrose	Total sugar	Hydro-lyzable polysac-charides
At time of picking.....	.. ..	12.19	11.5	2.43	5.52	8 24	0.32
Stored 10 days at 38° F. ...	Firm, sweet, good	11.10	11.2	2.47	5.20	7.88	0.27
Same, after 3 days at 72° F. ..	Slightly soft, overripe	10.84	11.8	2.53	4.61	7.38	0.24
Oiled, stored 10 days at 38° F.	Firm, sweet, good	11.15	11.5	2 57	5.07	7.91	0.26
Same, after 3 days at 73° F. ..	Firm, abnormal taste	11.30	11.3	2 70	5.60	8.51	0.24

The total sugar content apparently decreases slightly during cold
storage, and in the untreated melons it decreases still more after
removal to higher temperature. The latter change is much reduced
in the oiled melons, probably because the utilization of sugars in
respiration is retarded by the oil coating. The ratio of reducing
sugars to sucrose remains about the same in the stored fruit. Thus,
there can be no improvement in sugar content or in sweetness of
cantaloupes picked immature, during storage or shipment. These
results agree with those obtained on cantaloupes by Chase, Church

and Denny.[6]   Softening and loss of sugar by respiration are reduced by either cold storage or by oiling the surface of the fruit, but treatments of the latter type are likely to result in a product of undesirable flavor, in spite of the higher sugar content.

TABLE 6

CHANGES IN SOLIDS AND IN SUGAR CONTENT OF MELONS DURING STORAGE AT
20°–22°C. EXPRESSED AS PER CENT OF FRESH WEIGHT OF FLESH

Treatment	Quality	Total solids	Soluble solids	Reducing sugar	Sucrose	Total sugar
HONEY DEW (Series A)						
Picked Sept. 3	Hard, inedible	8 38	78 8	5 48	0 86	6 38
In ethylene 5 days	Rind yellowish, flesh soft, juicy .	8 31	84 3	3 62	3 25	7 00
Cellar 5 days.	Rind greenish, hard, flesh hard, inedible	8 12	82.4	4 87	1.71	6.60
Ethylene 5 days and cellar 7 days	Rind full yellow, flesh soft, juicy	8 49	85 6	2 93	4 06	7 20
Cellar 12 days	Rind greenish, hard, flesh firm, barely edible.	7.76	79 4	3.95	2 18	6 20
Ethylene 5 days and cellar 14 days.........	Rind yellow, soft, flesh soft, over-ripe	8.16	81 9	2 66	3.80	6 60
Cellar 19 days	Rind greenish, hard, flesh edible, insipid	7.40	81 7	4 01	2.06	6.10
CASABA (Series B)						
Picked Sept. 3......... ..	Rind greenish, inedible	7.45	74 4	4.58	0 78	5.40
Ethylene 5 days..... .	Rind bright yellow, flesh soft, edible	7.41	76.7	3 62	1 84	5 56
Cellar 5 days	Rind greenish, hard, flesh firm, barely edible...	7.46	74.9	4 58	0 79	5 41
Ethylene 5 days and cellar 7 days	Rind yellow, soft, flesh mushy, insipid	6.72	71.8	2 82	1 41	4.30
Cellar 12 days.	Rind partly yellow, flesh firm, barely edible...	6.92	71.0	3.93	0 90	4 88
Cellar 19 days.	Rind partly yellow, flesh slightly soft	6.69	72.9	3 78	1 00	4.83
Cellar 32 days.	Rind pale yellow, flesh firm, edible.	5 52	70 1	3 10	0 68	3.82
CASABA (series C)						
Picked Sept. 26.........	Rind partly yellow, flesh firm, barely edible......	8.56	81.7	4 01	3.37	7.56
Ethylene 2½ days....	Rind orange-yellow, flesh soft, juicy, good	8.70	81.0	3.18	3.87	7.26
Cellar 2½ days...........	Rind partly yellow, flesh firm, barely edible...	9.15	83.1	3.23	4.21	7.66
Ethylene 2½ days and cellar 7½ days..	Rind deep orange, flesh soft, over-ripe ....:.....	8.58	77.9	2.37	4.44	7.05
Cellar 10 days.............	Rind mostly yellow, slightly soft, good	8.84	78.8	3.12	3.66	6 97

Sugars in Other Melons During Storage.—Honey Dew and Casaba melons were harvested on September 3, the fruit being respectively 54 and 51 days old from date of setting.   The fruit was hard and the flesh inedible at this time.   It was realized that the sugar content was probably much lower than it would be in normally ripened fruit, but

it was thought advisable to study the changes during storage, of fruit that was picked unripe. The storage was in a cellar at 70° to 75°F. Since ethylene has been found to have a marked effect upon ripening processes of some other fruits, certain of the stored lots of melons were treated with this substance. The fruits of each variety were divided into two lots, and one lot was placed in a special chamber to which ethylene gas was added once each day for 5 days. After this period the ethylene-treated fruit was stored in the same room as the untreated. The concentration of ethylene used was 1 part to 2000 of air. The room was ventilated each day before adding the new charge of gas. Samples were taken for analysis from both treated and untreated fruit at the end of the 5-day period, and at intervals of 7 days thereafter. The changes in sugar content and in the color and texture of the fruit is given in table 6.

In the Honey Dews and in the Casabas of Series B, after 5 days' treatment with ethylene, the green color of the rind had completely disappeared, the former becoming creamy yellow and the latter bright yellow in color. At this time, the untreated fruits were about as green as when harvested. The flesh of the treated fruit was much softer than when picked, and could be classed as edible, although lacking in sweetness and flavor. The flesh of untreated fruit was still hard.

At the time of the later samplings, ethylene-treated fruit showed progressive softening, but with little improvement in flavor. They finally became so soft that the Honey Dews were classed as overripe 14 days after removal from the ethylene chamber, while the Casabas were overripe after 7 days. The untreated fruits remained firm a much longer time and though the changes in color and texture noted in the ethylene-treated fruit occurred in the untreated also, the changes were very much slower in the latter.

With regard to total sugar content, the samples taken at the 5- and 12-day periods after harvest show about the same amount as the freshly picked fruit. Samples taken still later show a marked decrease in the amount of total sugars, due no doubt to losses by respiration. There is a decrease in reducing sugars and corresponding increase in sucrose during storage of the Honey Dew and Casaba melons picked unripe, however. This change in form of sugar was also found to characterize the ripening process of fruits attached to the plant. The data also show that the change of reducing sugars to sucrose was much more rapid, and greater in amount, in the fruit of both varieties which was treated with ethylene, than in untreated fruit. This change may cause a somewhat sweeter taste in ethylene-treated melons.

Attention should now be called to the Casabas of Series C, in table 6. These were commercially picked fruits that from external appearances were about the same as the 53-day old fruit picked September 15 and discussed in table 2. In other words, these melons were in the stage considered "commercially mature" and suitable for shipment, though the rind was still hard and the flesh too firm to be palatable. Fruit of this series treated with ethylene at the rate of one part to 4000 of air, for 2½ days, was fully colored, with soft, juicy flesh of good eating quality. These melons had 40 per cent more sugar than those used in Series B, and this factor together with the rapid change in color and texture caused by the ethylene, gave them good eating quality. The fruit harvested at the same time, but untreated, also developed a high degree of edibility after 10 days' storage.

TABLE 7

PECTIC SUBSTANCES OF MELONS DURING STORAGE, EXPRESSED IN PERCENTAGE OF THE FRESH WEIGHT

Variety and treatment	Quality	Pectin	Insoluble pectic substances	Total	Per cent soluble
HONEY DEW (Series A)					
Picked Sept. 3...........	Hard, inedible ...	0 065	0 238	0 303	21 5
Ethylene 5 days..	Soft, juicy	0 141	0 115	0.256	55.1
Cellar 5 days	Hard, inedible	0.067	0 202	0 269	24 9
Ethylene 5 days and cellar 7 days........... .	Soft, juicy, fair flavor	0.175	0.075	0 250	70 0
Cellar 12 days...... . ..	Firm, barely edible.	0 114	0.146	0 260	43.8
Ethylene 5 days and cellar 14 days......	Overripe	0 149	0 118	0 267	55 8
Cellar 19 days...... . .	Firm, edible . . .	0 107	0.139	0 246	43 5
CASABA (Series B)					
Picked Sept. 3........ .. .	Hard, inedible	0 050	0 289	0.339	14 7
Ethylene 5 days.. .. .	Soft, edible	0 152	0 109	0 261	58 2
Cellar 5 days..	Firm, barely edible......	0 128	0.214	0.342	37.4
Ethylene 5 days and cellar 7 days	Mushy, insipid....	0 123	0.194	0.317	38.8
Cellar 12 days	Firm, barely edible	0 128	0.177	0 305	42 0
Cellar 19 days... . .	Slightly soft......	0 148	0.176	0 324	45 7
CASABA (Series C)					
Picked Sept. 26...........	Firm, barely edible......	0.105	0 162	0 267	39.3
Ethylene 2½ days......	Soft, juicy	0.190	0 110	0 300	63.3
Cellar 2½ days.	Firm, barely edible....	0.120	0.135	0.255	· 47.0
Ethylene 2½ days and cellar 7½ days...........	Soft, overripe	0.196	0.093	0 289	67 8
Cellar 10 days................	Slightly soft, good........................	0.143	0.127	0.270	53.0

Pectic Substances During Storage.—Samples from the same melons described in table 6 were also used for determination of pectic constituents, and the results are given in table 7. Here again the results

are given in terms of "pectin" in the water-soluble portion, and "insoluble pectic substances," the pectic acid and protopectin of the insoluble part of the sample.

During storage, fruit picked immature loses the green pigment of the rind, and the flesh becomes progressively softer and more juicy. It is seen from table 7 that the total of the pectic substances decreases slightly during storage, and that the pectin increases markedly at the expense of the insoluble fraction. The change to pectin from protopectin is in all cases much more marked in the fruit treated with ethylene than in the untreated. The rate and amount of this change appears to parallel the degree of softening of the fruit-flesh.

DISCUSSION OF THE EFFECTS OF ETHYLENE GAS

Tables 6 and 7 showed certain differences in the composition during storage of melons treated with ethylene and those untreated. Comparing fruit which had been in storage for the same number of days, ethylene treatment hastened removal of green pigment from the rind, and caused the flesh to become softer and more juicy. Ethylene also affected the normal rate of change in form of sugar and pectic substances. The course of these changes is shown in figures 3 and 4, for the fruit which was picked September 3, in a decidedly immature condition.

Figure 3 shows that the amount of total sugars may increase slightly during the first few days of storage, but remains nearly constant for the remainder of the storage period, with a slight tendency downward. The per cent of sucrose, however, increases markedly during storage, and this increase is more rapid and greater in final amount, in ethylene-treated fruit. The divergence of the curves for sucrose is most marked during the first period of 5 days during which the fruit was actually exposed to ethylene. In the next period, of 7 days, the increase of sucrose continues, but at a reduced rate, in both treated and non-treated fruit. The effect of the ethylene on conversion of reducing sugars to sucrose, seems to carry over beyond the period during which the fruit is actually exposed to the gas.

Figure 4 shows that while the total amount of pectic substances in the fruit decreases gradually during storage, the amount occurring in soluble form, as pectin, increases. As with sucrose, the increase in pectin is much greater in the early part of the storage period, when the fruit is treated with ethylene.

Since the change of protopectin to pectin in the fruit is supposedly due to the activity of the enzyme proto-pectinase, it is probable that the influence of ethylene upon this change in form of pectic materials, and upon the softening of the fruit, is due to the increased activation of this enzyme.
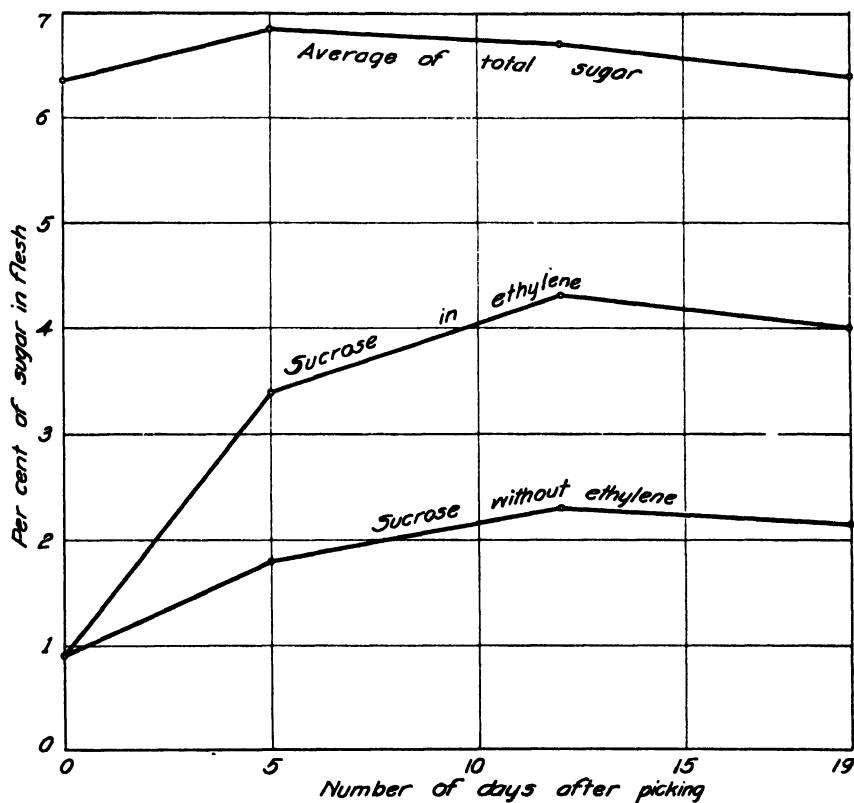
Fig. 3.—Sucrose content in Honey Dew melons during storage at 72°F, with and without 5 days ethylene treatment, compared to the average amount of total sugars in the two lots. (Compare with table 6.)

So far as the commercial utilization of ethylene on melons is concerned, it appears that it may well be used to hasten the softening, and to improve the edibility of melons picked in the commercially-mature stage, when these fruits are desired for immediate consumption. This means that its use will be limited to the grower who sells on local markets, rather than to the shipper. It is possible, too, that the receiver of California melons in eastern markets, can make use of the treatment, when the fruit arrives at destination in too green and hard

condition for immediate use. The use of ethylene will also be limited to Honey Dews and Casabas; there is no object in treating canta-loupes, for the types of changes accelerated by ethylene occur so rapidly in cantaloupes naturally, that nothing is to be gained by treating them. It should also be emphasized that while melons picked very immature may be softened, they are not given good edible quality by the ethylene treatment.
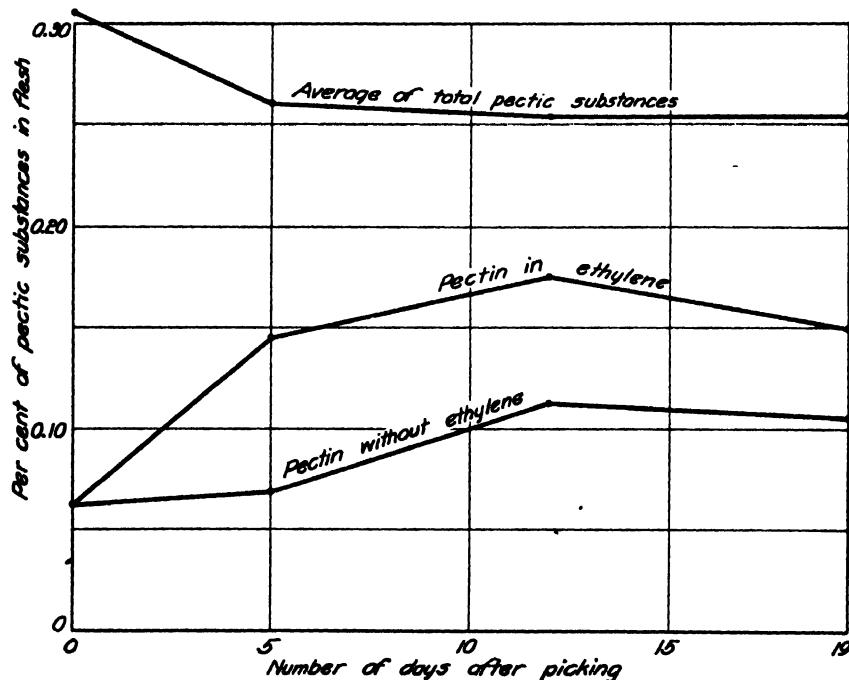
Fig. 4.—Changes in pectic substances in Honey Dew melons during storage, showing the relative increases in amount of pectin in fruit without ethylene and with 5 days ethylene treatment. (Compare with table 7.)

SUMMARY AND CONCLUSIONS

The late stages of development and the ripening process in canta-loupes, Honey Dews, Casabas, and watermelons, are characterized by the following changes when the fruits remain attached to the plant:

1. Progressive increase in per cent of total solids (dry matter), in total sugar content, in soluble solids, and in specific gravity of the juice.

2. Reducing sugars, which consist of approximately equal proportions of levulose and dextrose, decrease in amount during ripening, being partly used in respiration, and partly changed to sucrose.

3. Sucrose increases more rapidly than reducing sugars decrease, showing that sugars are moving into the fruit until the full-ripe stage is reached.

4. The total amount of pectic substances remains about the same, but the amount of protopectin, high in unripe melons, decreases rapidly during ripening, with a corresponding increase of pectin and probably also of pectic acid. The proportion of pectic substances in soluble form increases during ripening, suggesting that partial disintegration of cell walls is an important part of the ripening process.

5. The flesh becomes progressively sweeter and softer, and the rind turns from green to yellow.

Fruits which are picked from the plants in the immature condition show the following changes during the storage at ordinary temperatures (70°–75°F):

1. Little or no increase in sugar content, in the early part of the storage period, and generally a small decrease in sugars, during the latter part of storage, due to the losses occasioned by respiration.

2. Honey Dews and Casabas, during storage show the same change in form of sugars as do fruits attached to the plant, i.e., decrease in reducing sugar and increase in sucrose.

3. The total content of pectic substances decreases slightly, and protopectin is changed to pectin, just as in fruit attached to the plant.

4. The flesh becomes softer, but does not gain appreciably in sweetness, hence melons picked very immature, while the sugar content is low, upon artificial ripening become soft and to some extent juicy, but do not attain good flavor because of the lack of sugar.

Fruits of Honey Dew and Casaba picked slightly unripe and exposed to ethylene at the rate of 1 part to 2000 of air, or 1 part to 4000 of air, for 2 to 5 days, show no increase in sugar content, but

do show a marked acceleration in the rate of softening, in change from green to yellow color of the rind, and in conversion of reducing sugars to sucrose, compared to similar fruit stored without ethylene treatment. These changes do not result in palatable quality, if the fruit is picked so immature that its sugar content is low. But if the fruit is in the stage described in this paper as ''commercially mature,'' the treatment results in good eating quality in a much shorter time than is the case with fruit of the same stage of maturity not treated with ethylene.

The effects of ethylene upon the ripening process are believed to be due to activation of enzymatic reactions, and hence bring about changes in a short time that would ordinarily require a longer period for their accomplishment.

Pectic acid was found in the water extract of cantaloupes but not in that of Honey Dews or Casabas. Its occurrence in the cantaloupe only, may be due to the presence of an enzyme (pectase) in large amounts in that variety, resulting in rapid conversion of pectin to pectic acid during ripening or during the process of preparing the samples. This, together with the observed slower rate of ripening in storage of the Honey Dews and Casaba, suggest that these varieties differ markedly from cantaloupes in their enzyme activity, especially with regard to protopectin and pectin-hydrolyzing enzymes. With this difference in enzyme activity, it is possible that long-keeping varieties of cantaloupes can be developed by the plant breeder.

Both pectic acid and protopectin were found in the insoluble residue of the four kinds of melons examined. However, the relation of these substances to each other was not constant, and did not show a consistent relation to the ripening process.

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BORON IN THE SOILS AND IRRIGATION WATERS OF SOUTHERN CALIFORNIA AND ITS RELATION TO CITRUS AND WALNUT CULTURE[1]

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INTRODUCTION

In the latter part of 1925 and at various times subsequently, our attention has been called to certain citrus trees growing in the vicinity of several different citrus packing houses in southern California that have been injured severely. Usually the injury developed rather suddenly. In some instances only a few trees, in others several hundred, have been affected. In practically every case the trees were previously vigorous and thrifty.

The injury first became apparent by a yellowing of certain parts of the leaves, usually beginning with the tips and margins, and this was soon followed by a similar yellowing of the tissues between the veins. As the effect progressed the tips died back. Sometimes isolated areas along the margins of the leaves or small spots between the veins were killed. Many of the affected leaves fell prematurely and not infrequently the smaller twigs died. The more severely affected trees shed practically all of their leaves and in a few instances the entire tree died. The shedding of the leaves of the less severely affected trees was followed sooner or later by the development of new shoots

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whose leaves appeared normal for a few weeks save for a slight paleness and perhaps a reduction in size, but later the new shoots went the way of their predecessors.

The injury referred to above has been chiefly confined to orange trees. In a few instances lemon and walnut trees have also been affected. The walnut leaves turned brown around the margins and died back from the tips, the injury gradually extending into the mesophyll tissues toward the midrib. The margins of the affected tissues were usually irregular, and not infrequently brown spots of various sizes developed between the veins. Many of the walnut leaves fell prematurely, but usually the injurious effect was not apparent until after midsummer.

Soon after this problem came to our attention it was found that the injury in question was associated with a contamination of the irrigation water brought about by the accidental discharge of wash-water from nearby citrus packing houses. These wash-waters frequently contained dissolved boron compounds, either borax or boric acid.[4] Chemical analysis showed positive relationships between the injury to the trees and the water-soluble boron content of the soil, on the one hand, and the boron content of the leaves, on the other.

It was noted that the appearance of the less severely affected lemon and walnut trees of these groves was markedly similar to that of certain abnormal lemon and walnut groves of Ventura County which had been under investigation for several years, but the determination of the cause of which has baffled all previous study. Recently we have found that the abnormality of these, as well as of certain groves in several other localities, is related to the occurrence of soluble boron, which occurs either as a natural constituent of the irrigation supply or of the soil itself.

It is well known that several species of plants show marked response to the presence of soluble boron in the nutrient medium. On the one hand, the investigations of Agulhon,[1] Mazé,[11] Warington,[14] Brenchley and Thornton,[3] Brenchley and Warington,[4] and Sommer and Lipman[13] have clearly established the fact that boron is essential for the normal functioning of certain plants. Small amounts of this element now appear to be just as necessary for the complete development of these plants as nitrogen, phosphorus, potassium, or calcium,

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[4] A concentrated solution of borax or boric acid is used as a fungicide in many of the citrus packing houses of California. After a given solution has been used for a certain length of time it was formerly discharged into a convenient drainage channel. However, after it became established that boron is extremely toxic, the packing house waste has been disposed of in such way as to avoid the contamination of irrigation supplies and the impregnation of orchard soils.

which elements have long been known to be essential. On the other hand, Hotter,[9] Agulhon,[1] Brenchley,[2] Warington,[14] Conner and Fergus,[6] Skinner *et al.*,[12] Collings,[5] and others have found that toxic effects appear if the concentration of boron exceeds a very low value. Some of these workers found that boron is extremely toxic, an application at the rate of only a few pounds per acre being sufficient to produce marked injury to certain plants.

In an address delivered before the Washington Academy of Sciences in 1920, Kellerman[10] suggested that toxic concentrations of boron are likely to be found here and there in the soils and irrigation waters of the southwestern part of America. He pointed out that large deposits of borax and other boron minerals occur in several places in southern California and that analysis has shown that small amounts of boron occur in various lakes and streams of this region. Dr. Kellerman also made the interesting suggestion that the high toxicity of certain alkali soils of the semi-arid region of America may be due in part at least to soluble boron.

THE EFFECT OF BORON ON CITRUS AND WALNUT TREES

We have found that boron is widely distributed in the soils of southern California.[5] As shown in table 1, the highest concentration of water-soluble boron was found in the soil from those orchards where certain fruit trees show definite injury. Moreover, the results obtained by analyzing the leaves of citrus and walnut trees have shown a striking relationship between their boron content and their general state of health. The boron content of the normal leaf of these species was found to vary from only a few parts per million to approximately 100 p.p.m. (table 2). The leaves of the injured trees, on the other hand, contain much more boron, the amount found ranging from 266 to 1,679 p.p.m. (table 3).

[5] Boron was determined by the well-known method of distillation with methyl alcohol, followed by double titration of the distillate with standard alkali, using paranitrophenol and phenolphthalein as indicators and mannitol as catalyzer. The entire process was carried out in boron-free apparatus and with the use of boron-free reagents. With soil the determination was usually made by leaching a kilogram with 2000 cc distilled water, evaporating the leachate to dryness in silica dishes, transferring the residue to a boron-free flask and distilling with methyl alcohol in the presence of phosphoric acid. With irrigation water 4 liters was evaporated to dryness and the residue was then subjected to the same process of distillation. With plant material 10 to 20 grams of the dry substance was ignited in a platinum dish in the presence of an excess of lime. The char was leached with dilute hydrochloric acid, the leachate made alkaline, and the determination was then completed as in the case of the soil extracts. Duplicate determinations were always made.

TABLE 1

WATER-SOLUBLE BORON CONTENT OF SOILS. BASED ON THE DRY WEIGHT

Locality	Evidence of injury	Source of boron	Boron content (p. p. m.)
Riverside	None		Trace
Riverside	None		Trace
La Habra	None		Trace
Tustin	None		0.3
North Pomona	Positive	Packing house wash-water	5.2
Seape Canyon	Positive	Irrigation supply	7.7
Seape	Positive	Irrigation supply	3.0
Bardsdale	Positive	Irrigation supply	3.6
Tustin	Positive	Irrigation supply	2 3
San Fernando Valley near Tujunga Canyon	Positive	Irrigation supply	5.1
Santa Paula	Positive	Native to soil	3.1
Chula Vista	Positive	Native to soil	4.0
Oasis	Positive	Native to soil	21.0

TABLE 2

BORON CONTENT OF NORMAL CITRUS AND WALNUT LEAVES. BASED ON THE DRY WEIGHT

Kind of leaf	Locality	Boron content (p. p. m.)
Orange	Arlington	46
Orange	Villa Park	87
Orange	Anaheim	81
Orange	Arlington	30
Orange	Cucamonga	30
Orange	Redlands	44
Orange	Redlands	25
Orange	Upland	24
Orange	Ontario	21
Lemon	Arlington	27
Lemon	Bloomington	19
Lemon	Upland	27
Lemon	Tustin	54
Lemon	Ontario	25
Walnut	Whittier	93
Walnut	Whittier	103
Walnut	Santa Ana	51
Walnut	Anaheim	112
Walnut	Puente	16
Walnut	Hemet	38
Walnut	Corona	26
Walnut	La Habra	61
Walnut	Olive	54

Experiments with Artificial Applications of Boron.—The relationship found between the injury to the citrus and walnut trees and the boron content of the soil and of the leaves indicated strongly that the observed injury was produced by boron. This view has been strengthened by the results of tests made by applying known amounts

TABLE 3

BORON CONTENT OF CITRUS AND WALNUT LEAVES THAT WERE INJURED BY WASTE WATERS FROM PACKING HOUSES. BASED ON THE DRY WEIGHT

Kind of leaf	Locality	Boron content (p. p. m.)
Lemon	North Pomona	407
Lemon	North Pomona	266
Lemon	Glendora	839
Orange	Glendora	1,679
Orange	Glendora	1,385
Orange	Azusa	1,281
Orange	Azusa	756
Orange	Covina	900
Walnut	North Pomona	456
Walnut	Ontario	683

of boron to the soil or to the water used in irrigating healthy trees. In one experiment, lemon trees about twenty years of age were irrigated at monthly intervals with water containing approximately 5, 25, and 50 p.p.m. of boron respectively, applied as ordinary borax. The experiment was begun in March, 1926, at the Citrus Experiment Station on soil of a sandy loam type.

Where the water containing 50 p.p.m. was used, practically every leaf fell from the trees within a few days after the second application was made. The trees that were irrigated with the solution containing 25 p.p.m. began to show symptoms of leaf injury within about three months. The leaves turned yellow around the margins and between the veins. The tips gradually died back and many of the leaves fell. The water which contained only 5 p.p.m. of boron produced no apparent effect until after the fifth application. At that time, however, the leaves became markedly affected. The appearance of these leaves was indistinguishable from that of the trees that had been injured by boron waste water from the various packing houses.

An experiment conducted at our request by Mr. C. A. Jensen with mature lemon trees growing on a comparatively heavy type of soil at the Limoneira Ranch, Santa Paula, California, yielded similar results. Where he made the smaller applications of boron, injury set in only after a greater number of applications than was the case on the lighter type of soil at Riverside. The appearance of the leaves was essentially the same, however. The general nature of the effect was identical with that produced at Riverside.

As is shown in table 4, the injury to lemon and orange trees, produced by the application of known amounts of boron, was accompanied

by the absorption and deposition in the leaves of abnormal quantities of boron, just as was found to be the case with the groves referred to above.[6]

TABLE 4

BORON CONTENT OF CITRUS LEAVES AS AFFECTED BY ARTIFICIAL APPLICATIONS OF
BORAX. BASED ON THE DRY WEIGHT

Kind of leaf	Locality	Condition	Boron content (p. p. m.)
Orange	Riverside	Normal*.	43
Orange	Riverside	Injured	463
Lemon	Riverside	Normal*.	76
Lemon	Riverside	Injured	308
Lemon	Riverside	Severely injured	1,083
Lemon	Limoneira	Injured	545
Lemon	Limoneira	Severely injured	1,400

* Samples taken from untreated trees.

The Natural Occurrence of Boron.—As has been pointed out already, our attention was drawn to the fact that there is a marked similarity between the appearance of the lemon trees injured by waste waters from packing houses and certain lemon groves located in Ventura County, California. Investigation has shown a definite relationship between the occurrence of these symptoms and the use of certain irrigation supplies. One of these supplies of irrigation water is drawn from the Sespe Creek and another from wells located near Fillmore. Several citrus orchards near Piru, whose irrigation supply is drawn in part from the Piru Creek, present a similar appearance, especially in the case of lemon trees. Certain citrus and walnut groves located on the south side of the Santa Clara River between points approximately opposite Santa Paula and Fillmore, and a few relatively small areas on the north side of the Santa Clara River, both east and west of Santa Paula, also show evidence of boron injury. Minor indications of boron toxicity are also found in the various lemon and walnut groves of other parts of Ventura County. For example, several walnut groves found between Saticoy and Ventura have shown slight boron injury during the latter part of each summer for several years. Similar conditions exist in certain walnut groves of the Santa Susana Valley.

Samples of leaves taken from the above-named localities were found to contain excessive amounts of boron (see table 5). As suggested already, the chief source of the boron in these localities appears

[6] A more detailed discussion of the toxic effect of boron, based on culture experiments with citrus and walnut trees, will be presented in a separate paper by A. R. C. Hass.

to be the irrigation supply. As shown in table 6, several irrigation waters of this general section contain appreciable amounts of boron. This is especially true of the Sespe and Piru creeks. Analysis of the water from various wells indicates that a considerable portion of the

TABLE 5

BORON CONTENT OF INJURED CITRUS AND WALNUT LEAVES FROM VENTURA
COUNTY. BASED ON THE DRY WEIGHT

Kind of leaf	Locality	Boron content (p. p. m.)
Lemon	Sespe	378
Lemon	Sespe	651
Lemon	Sespe Canyon	796
Lemon	Sespe Canyon	927
Lemon	Santa Paula	495
Lemon	Piru	760
Orange	Piru	1,111
Orange	Sespe Canyon	812
Walnut	Sespe Canyon	1,018
Walnut	Ventura	365
Walnut	Ventura	360
Walnut	Santa Susana	469
Walnut	Moorpark	570

TABLE 6

BORON CONTENT OF IRRIGATION WATERS

Source	Date	Boron content (p. p. m.)
Sespe Creek	June, 1926	3 3
Sespe Creek	April, 1927	1.7
Sespe Creek	Sept., 1927	1 9
Piru Creek	Nov., 1926	1 3
Santa Clara River near Santa Paula	June, 1926	0 4
Santa Paula Creek	June, 1926	0 2
Well No. 1, Santa Clara Valley	Nov., 1926	3 6
Well No. 1, Santa Clara Valley	May, 1927	1 2
Well No. 1, Santa Clara Valley	Sept., 1927	1 7
Well No. 2, Santa Clara Valley	June, 1927	0 9
Well No. 3, Santa Clara Valley	June, 1927	0 5
Well No. 4, Santa Clara Valley	June, 1927	0.6
Well No. 5, Santa Clara Valley	June, 1927	0 9
Well No. 6, Santa Clara Valley	Aug., 1927	1.0

underground water supply of the Santa Clara Valley contains somewhat more than 0.5 p.p.m. of boron. In a few comparatively small areas of this valley abnormal amounts of soluble boron occur, probably as a natural constituent of the soil. Samples of soil from one of these spots showed 6 p.p.m. and from another 5 p.p.m. of water-soluble boron.

The fact that small amounts of soluble boron occur in the soil and water of this section is not surprising in view of the geological conditions. Deposits of colemanite, a calcium borate, outcrop at certain places in the watershed north of the Santa Clara Valley. One of these deposits was formerly drawn upon as a commercial source of borax. It is reasonable to expect that the water draining from such a watershed, and the soil that has been derived in part from formations which contain colemanite, would contain appreciable quantities of boron.

For the past two years certain lemon and walnut groves growing on the lighter types of soil in various parts of the San Fernando Valley have shown typical symptoms of boron injury. The appearance of the leaves is indistinguishable from that produced by artificial

TABLE 7

BORON CONTENT OF LEMON AND WALNUT LEAVES FROM SAN FERNANDO VALLEY. BASED ON THE DRY WEIGHT

Kind of leaf	Locality	Boron content (p. p. m.)
Lemon	Tujunga	417
Lemon	San Fernando	550
Lemon	San Fernando Heights	441
Lemon	Pacoima	381
Walnut	San Fernando	380
Walnut	Owensmouth	397
Walnut	Zelzah	278

applications of boron. Analyses of leaves from these trees, reported in table 7, showed a relatively high content of boron. It is probable that the source of boron in this instance is the Owens River, from which the irrigation supply is drawn. Samples of this water taken near the San Fernando reservoir on October 1, December 1, and December 22, 1926, showed 1.9, 1.2, and 1.0 p.p.m. of boron, respectively. It is probable that samples taken at other times will show still greater variation in boron content, since wide variation in the content of other soluble constituents is known to characterize the water of various streams of the semi-arid region.

It has been found that boron toxicity also occurs in certain grapefruit orchards near Oasis in the Coachella Valley. In this locality the injury to grapefruit trees becomes most apparent in the late fall and winter months. The affected leaves practically all fall off during the winter and a profuse new growth of normal appearance develops the following spring. In this instance the boron is probably a native

constituent of the soil. Mild boron injury is also shown by grapefruit trees growing in certain parts of the Imperial Valley.

A relatively large citrus orchard composed of both lemon and orange trees, located about five miles southwest of Tustin, has been injuriously affected by boron. The source of the boron in this case appears to be the well from which the irrigation supply is drawn. This water contains about 1 p.p.m. of boron. There is also slight indication of boron injury in a few lemon groves near Chula Vista in San Diego County and in certain walnut groves located near Goleta in Santa Barbara County.

GENERAL DISCUSSION

Normally a given leaf of the lemon or orange remains attached for thirty to forty months, and the natural abscission incident to senility occurs at the base of the petiole. On the other hand, when severely injured by boron the leaves may fall at the age of six to ten months, and in this case the abscission sometimes occurs at the upper end of the petiole.

Although injurious amounts of boron may be present in the nutrient medium, the new growth may not show any indication of injury for several weeks. A little later, however, the leaves of this growth will manifest typical symptoms of boron injury. Many of these leaves fall the following winter; consequently the foliage in the interior of the tree becomes thin.

The lemon tree shows the toxic effect of boron in a characteristic way; experience will enable anyone to recognize the symptoms readily. Frequently the symptoms are shown on the foliage of the interior as well as of the exterior of the tree. The walnut may not show the toxic effect until past midsummer, but by August or September the leaves turn brown around the margins, gradually die back, and fall off prematurely.

According to our observations, lemon, grapefruit, and walnut trees are especially sensitive to boron. The orange is somewhat less sensitive. At present it is not possible to say just what is the minimum concentration of boron that will produce injury to these species. It seems certain, however, that an irrigation water which contains 1 p.p.m. of boron will ultimately produce more or less injury.

If the concentration of boron is not too high and yet sufficient to produce injury, most of the older leaves of citrus trees fall off during

the latter part of the winter and early spring months. This as stated above is often followed by the development of numerous new shoots which for a few weeks seem to be approximately normal, and the new growth is likely to give the impression that the cause of the injury has been removed and that the trees are on the way to recovery. However, with the approach of the following fall typical symptoms of boron injury again appear and by winter practically every leaf will show pronounced discoloration. These leaves fall a few months later and again new shoots appear the next spring. Lemon and orange trees may continue this sort of an existence for several years. However, the fruit that is produced is inferior in quality and the crop is small.

Although certain irrigation waters which contain approximately 1 p.p.m. of boron have produced marked toxicity, the injury did not become apparent until after these waters had been in use for several years. In these cases two sets of factors are probably involved: first, the absorptive power of the soil, and second, concentration due to evaporation. The former tends to delay the development of injury, the latter operates to accentuate it. As stated already, with the same concentration of boron in the irrigation water, toxic effects develop sooner on light than on heavy soil, but it is not safe to conclude from this fact that an irrigation water that contains a small amount of boron can be used indefinitely on a heavy type of soil. Sooner or later the soil will become saturated with boron and the concentration resulting from evaporation may then be expected to bring about injury.

Laboratory studies have shown that soluble boron can be leached out of the soil. In an experiment with soil samples taken from a badly affected lemon orchard of the San Fernando Valley it was found that practically all of the soluble boron was removed in the early stages of the leaching. This experiment helps to explain the fact that the boron-affected orchards commonly show the most pronounced injury in the fall and winter months. The boron which has accumulated during the previous irrigation season is probably leached out to some extent by the relatively heavy winter rains which occur in this section. In this way the accumulation of boron is automatically held in check.

It is possible, therefore, that an occasional heavy irrigation applied by the flooding method might leach out the boron which accumulates as a result of evaporation and thus prove to be distinctly helpful as a means of reducing the injury. In this connection it is important to bear in mind that the soil moisture inevitably becomes more concen-

trated with respect to boron as well as other salts than the irrigation water itself, owing to evaporation. The results of a simple experiment will serve to illustrate this point. Samples of soil were taken from two lemon groves of the San Fernando Valley which showed definite boron injury. The soil solution removed from these samples by the displacement method was found to contain 6.0 p.p.m. in one case and 6.5 p.p.m. in the other. However, analysis of the irrigation water used regularly on these groves revealed only·1 p.p.m. of boron.

The results of this investigation strongly indicate that boron is readily absorbed by citrus and walnut trees and that this element tends to accumulate in the leaves of these species. Because of this fact a determination of the boron content of the leaves affords a valuable indication as to the boron conditions in the soil. As stated already the boron content of the dry matter of normal citrus and walnut leaves, when grown in southern California, ordinarily does not exceed 100 p.p.m. Frequently it is less than 50 p.p.m. On the other hand, leaves that show definite boron injury are likely to contain several hundred parts per million of boron. We have taken advantage of this fact in deciding whether boron is causally related to a given abnormality of these trees.

In this connection it is important to state that the age and stage of development of the leaf has an important bearing on its content of boron. Apparently the absorption of boron is a very gradual process, and it is not until the concentration within the cells of the leaf exceeds a certain point that toxic effects become manifest. Relatively mature leaves should therefore be chosen for analysis. Whether other species of fruit trees absorb boron in a similar way has not yet been determined.

On the basis of our analyses small amounts of boron appear to be widely distributed in southern California and the citrus and walnut trees of this section always contain appreciable amounts of this element. Whether a small amount of boron is essential for these plants,[7] as is the case with several other species, cannot now be definitely stated. At any rate it is safe to say that boron is toxic if present in more than a very low concentration. In certain places there appears to be an association between boron and a type of abnormality of citrus trees closely resembling, if not identical with, the well-known condition usually designated as 'mottle leaf.' It is possible that by careful study of the effect of boron, important light may be thrown on the mottle-leaf question.

Although the toxic effect produced by boron is characteristic and ordinarily it can be easily recognized, Haas and Thomas[8] have pointed out that boron symptoms may be confused with those produced by an excess of sulfate. The fact that sulfate is a predominant constituent of the irrigation water of certain localities should therefore be taken into consideration.

It seems desirable to emphasize the fact that our knowledge regarding the rôle of boron in the nutrition of plants, as well as that of various other elements, is inadequate. During recent years various workers have shown that small amounts of boron, and of several other elements which are not commonly considered to be essential to plant growth, play an important part in the development of various species. Usually these elements are required in very small amounts and some of them ordinarily occur in the soil in mere traces. It is a curious fact that, although small amounts of such elements as boron, manganese, copper, zinc, etc., are required for the full development of various plants, every investigator who has studied this question has noted that toxicity results when the concentration exceeds a very low level. In this respect these elements differ greatly from such elements as nitrogen and calcium. It is certain that a knowledge of the function of the above-named elements will be helpful in the study of the nutritional processes of various fruit trees. Such knowledge may prove to be of aid in the practical solution of some of the nutritional difficulties that are encountered in many places in California.

SUMMARY

1. Concentrations of boron occur in certain irrigation waters of southern California that are toxic to citrus and walnut trees. In a few relatively small areas the soil contains an injurious quantity of soluble boron, which has probably accumulated as a result of purely natural causes.

2. Citrus trees show the toxic effect of boron by a yellowing of the older leaves around the margins and between the veins and a dying back of the tips and margins. The new growth may not show the injury until it is several months old. Many of the affected leaves fall off in the winter and early spring months. When walnut trees are injured by boron, the leaves turn brown around the margins and between the veins during August and September. Earlier in the year the leaves may not show any evidence of boron injury. Boron-affected walnut leaves tend to fall prematurely.

3. Citrus and walnut trees absorb boron readily and this element tends to accumulate in the leaves. The determination of the boron content of the leaves of citrus and walnut trees gives valuable indication as to whether this element is the cause of abnormal leaf conditions.

4. When introduced into the soil as a constituent of the irrigation water, boron gradually accumulates in the upper layers of the soil as a result of evaporation. Heavy rains probably carry down some of the boron from time to time and thus retard the accumulation of soluble boron in the region of tree roots.

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FACTORS AFFECTING SELLING PRICES OF LAND IN THE ELEVENTH FEDERAL FARM LOAN DISTRICT

DAVID WEEKS[1, 2]

INTRODUCTION

Farm land valuation is a subject which is of interest to farmers; to other buyers and sellers of land; to the accountant who would apportion costs and income among the factors of production; to the mortgage holder; to the tax assessor; to the economist studying problems of land utilization, farm organization or agricultural relief; to the congressman who would improve our public land policies; to the chamber of commerce interested in regional planning; and to the farm real estate broker whose living depends upon his knowledge of values. "At the outset let it be understood that the subject of land valuation is little explored. Economists and real estate appraisers are still feeling their way toward guiding principles."[3] Before it is possible to place reliable principles and methods of appraisal in the hands of practical minded appraisers, farmers and real estate dealers, it is necessary to develop fundamental truths regarding land valuation. To make possible the proper interpretation of the great unorganized mass of knowledge pertaining to the subject, it is necessary to use methods which have been developed by economists and statisticians.

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[3] Ely and Morehouse. Elements of land economics. p. 236. The Macmillan Co. 1924.

This discussion is prepared primarily for the use of economists and statisticians working on this complex problem. When the field has been more thoroughly covered, statements can be prepared of the findings which will be much more useful to those desiring to apply them directly to problems in the field. In the meantime, the material as here presented contains information which should clarify some of the difficulties of appraisal. It might be well to caution those unaccustomed to the terminology used in parts of this paper to be patient with the instruments employed while they are making use of such material as may be gleaned from the more readable portions.

Current Methods of Farm Land Valuation.—A certain bank president speaking to a group of bankers stated that, after all, farm land appraisal is a "good guess," with all the data before you. This same bank president is noted for his use of scientific knowledge when it can be demonstrated that it might increase the accuracy of the work of his appraisers. Taylor[4] uses the principle of capitalization as the basis of his discussion of land valuation calling attention to some of the limitations of this method. Ely and Morehouse[5] state that the income of land is the basis for arriving at its value and that the process of capitalizing land income into a capital value is the "heart of the problem of land valuation." Recognizing capitalization as the heart of the problem, however, does not deter these authors from stating their position in regard to the limited usefulness of this method as the basis of appraisal. "Having laid down the principle underlying land values," they state, "we are ready to depart from it! We have assumed several figures and regarded them as fixed, whereas, in reality they are highly variable in time and place."[6]

The general method used in land valuation by the Interstate Commerce Commission is summarized in the following paragraph:

"In determining the unit of value for the zone, the appraiser will generally be governed by the sale, assessment, and opinion data. He should not, however, fall into the habit of slavishly following the average of the information obtained. The object in procuring sales, assessments, and opinions is that the appraiser may be informed as to values. The end sought is the value of the land, not the average of the data. One particular sale, known to be characteristic, might outweigh three or four other sales or a mass of other data."[7]

[4] Taylor, Henry C. Outlines of agricultural economics. pp. 250–263. The Macmillan Co. 1925.

[5] Ely and Morehouse. Elements of land economics. pp. 239, 242. The Macmillian Co. 1924.

[6] *Ibid.*, p. 244.

[7] Artuad, T. P. Instructions pertaining to land appraisals. Interstate Commerce Com. Bureau of Valuations. p. 7. Government Printing Office, Washington, D. C. 1922.

That those engaged in practical land valuation have had to depart from capitalizing land income as a basis of valuation is illustrated in the case of values placed on undeveloped railroad land by one of the large land-holding railroad companies. "We watch the land market," says the supervisor of land sales for this company, "and place values on our lands according to the demand, making allowances, of course, for differences in topography, location, etc." Foreclosures, delinquencies, complaints, loss of business, all play an important part in the appraisal policies of banking institutions. Trial and error are the basis of the "good guesses" of values placed upon farms by the practical appraisers of these institutions. Between a high rate of foreclosures and delinquencies, on the one hand, and complaints of applicants for loans and actual loss of business on the other, the land appraiser works, using his best judgement, in the placing of values. The man of experience with a knowledge of soils, crops, crop diseases, irrigation and drainage, will do a pretty good job of guessing. No amount of technical investigation will ever replace entirely the judgment of such a practical man of experience. If, however, we can add to his knowledge by the study of relationships between some of the important qualities of land and their effect upon value, we may in time be able to increase the efficiency and the accuracy of his work.

Value or Price?—Those who place all of their faith upon capitalization of net income as a basis of value are able to think in terms of value entirely apart from price. Those whose interest in land value is entirely an evaluation of the security for the purpose of a loan, insofar as they are concerned about the ability of the farmer to make payments on interest and principal when due, are also able to think of land value entirely apart from price. The real security value, however, of a farm is its most probable selling price, taking into consideration possible changes in economic conditions. A margin must also be allowed for costs of foreclosure. A farmer buying a piece of land thinks of the value in terms of the price he must pay for it, the price he might be able to sell it for, and also in terms of the income he expects to get from it. The dealer in land thinks wholly in terms of purchase and selling prices. The economist thinks of land value as capitalized rent and a result of the difference between cost and income rather than as one of the costs. The accountant thinks of land value as so much invested capital for which interest must be paid as one of the costs of operation. The present study is an analysis of prices at which farms have actually exchanged hands and it will be assumed by the writer that the price at which any particular farm

changes hands is not necessarily its value but that the most probable
price at which that farm would sell on the market is its market value,
and that the "level," or line of trend, of prices about which this most
probable value fluctuates over a period of time will be considered as
the normal value, "the term normal being taken to refer to a long
period of time."[8] This does not preclude the use of income data as
an index of the normal or the market value. The problem is so
complex that it will be necessary to approach it from many angles.
This report necessarily covers only a portion of the entire field.

Income and Selling Prices of Land as Measures of Value.—There
are many difficulties to be met in the appraisal of land, either on the
basis of capitalizing net income or on the basis of measuring values by
the use of established relationships between selling prices and land
qualities. Appraisal by either method requires a study of those
elements which cause different farms to have different values.

Although the research reported in this publication has not covered
factors of net and gross income as affecting land value, it should not
be assumed without further investigation that similar studies cannot
be made of the relationships between net and gross income and the
same land qualities which have been correlated in this analysis with
selling prices. Difficult as income is to determine, careful correlation
of net income and land qualities may result in measures which may
be used as indexes of value. Productivity and income should enter
into the problem, not so much in the form of complete estimates of
net income to be capitalized, as in the form of indexes of those qualities
of land which are the causal factors of that net income.

Disadvantage of Appraisal on the Basis of Net Income.—Income
from land is practically inseparable from the income of other elements
of production; namely, labor, equipment, and management. Changing
proportions of equipment, labor and land result in changing propor-
tions of the total product due directly to the product of the soil; there-
fore, the income due directly to the land may be an ever changing
quantity and impossible to determine. Income from land is constantly
changing because of climatic and crop conditions, changing prices, and
changing costs of production. The changes in land income vary over
different areas at different rates. It is difficult to determine yields.
The experience of one year is not sufficient. Information for a num-
ber of years can seldom be secured with accuracy. Translation of
yields into net income is a difficult accounting process. It is seldom
done with accuracy on account of the amount of labor involved and

⁸ Marshall, Alfred. Principles of economics. p. 371. The Macmillan Co., 1920.

the need of training in farm accounting methods. The limited accuracy of available data makes impossible accurate estimates of net income. In a new region prices are not established, productivity of land is not established, crop adaptation is uncertain and the whole basis of land valuation hangs upon the future development of the surrounding country. This difficulty is common to all methods of land valuation. In evaluating land which has not been developed, there is no means, except by comparison with other farms, to determine the potential producing power. A rational basis for doing this may have been developed by some appraisers, but in most cases guess work under the name of "judgment" is the only basis of making this important step in the process of appraisal. If income can be determined, its capitalization into value is dependent upon the selection of "the current rate of discount." The current rate of discount may vary between wide extremes according to the character of the security, the condition of the money market, and the personality of the borrower. In general, income from land tends to increase while income from capital tends to decrease. This would cause an ever increasing divergence between the rate used in capitalization and the actual land income.

Finally, income from land is not the only factor determining land value. Not even when all of the indirect sources of income are considered does the income necessarily indicate value, if we are to define value as the most probable selling price. Alternative opportunities in other business is often an important influence in determining the demand for land. There is a resulting effect upon land price.

Difficulties Encountered in Making Sales Price the Basis of Land Appraisal.—In the case of appraisal on the basis of sales price analysis, classification of land is difficult owing to the great variability in its character. Market price of land changes as do prices of other commodities. These changes take place in response to changes in demand, arising from varying demand for agricultural commodities; from business conditions which, at different times, cause men to seek employment in agriculture because of the difficulty in finding employment elsewhere; and, from changes in the money market and credit conditions, which may to some extent affect the rate of land purchase because of the varying degree to which money is available for such purchase. Standards set temporarily by land sales agencies combined with the general lack of knowledge on the part of many purchasers concerning the value of the land they are buying, and uncertainty as to actual net rates, may, for a time, upset the economic trend of land

prices, as in the case of appraisal by capitalizing net income. In a newly developed region, sales prices are likely to be unstable because of uncertainty as to the future development of that region and the marketability of crops. The best adaptability of the crops to the various soil conditions must also be determined by experience, to a large extent. Soil surveys and climatological data are reducing the uncertainty which existed in early settlements. However, many do not avail themselves of these modern facilities for obtaining information. One of the most important difficulties in both the selling price and net income analyses for obtaining land values, is the inaccuracy of data and lack of reliable basic information.

Purpose of the Analysis.—The purpose of the present study has been to determine quantitative relationships between selling price of land and the factors that affect that selling price, with a view to working toward a basis of more rational farm appraisal, in which quantitative measurements of land qualities may, in part, take the place of rough estimates of the degree to which different land qualities affect value. The objective of the entire research has been to ascertain how certain land qualities, which are variables, are associated with sales prices, that is to determine what men are willing to pay for certain land qualities—those land qualities which can be measured in degree, or quantity. An endeavor has been made to find out how soils and temperature combinations may be measured in terms of relative productivity, how crop value per acre may be expressed upon a relative basis. Size of farm can be expressed in acres, value of buildings in dollars, productivity in yield per acre, etc. It is not expected that the results of this work will revolutionize appraisal methods, but that the first results will help research worker better to understand the relative importance of different land value elements. As progress is made in the years to come and more data become available which are applicable to such studies, it is believed that actual measurement can be made of the effect of certain elements upon value, which are at present determined by rough approximations. At such time as confidence is established in the results, the necessarily complex methods may be reduced to tables and simple methods for the use of appraisers and others.

The Scope of the Study.—The multitude of complications which would arise if all kinds and classes of farm land were included in the investigation, especially in the beginning, make it necessary to limit the scope of the study. Such an analysis naturally divides itself into two important divisions. The first of these is a consideration of the

dynamic economic factors which influence the general level of land prices while the second phase of the research inquires into the factors which cause differentials in prices of different farms. In the first case, it is possible to include large numbers of farms of different kinds and sizes for the purpose of drawing general conclusions concerning the relationships between indexes of economic conditions and price of land. Even this study, however, must be made with the fact in mind that farms of different types react differently to given economic conditions. In the analysis of the second phase the scope of the work must be radically reduced, first to get a starting point for use as a basis of comparison, and second to reduce the amount of work to a volume within the limit of possible accomplishment. In the study of differentials in price, therefore, the work has been narrowed down to an inquiry into the causes of differences in prices of dairy farms with the expectation that the results obtained will be useful in extending the analysis to other types of agriculture. Not only has it been necessary to reduce this phase of the investigation to one type of agriculture, but it has also been necessary to exclude a large number of variables such as poor irrigation and drainage conditions, excessive alkali, hardpan, weeds, pests, and other characteristics which would make the number of variables so large that the analysis would be too cumbersome. As a starting point, therefore, farms of almost ideal physical conditions have been selected for the purpose of developing indexes to be applied later in the evaluation of some of these more difficult factors of a heterogeneous character. This process of elimination has reduced the number of cases available for correlation studies to a rather small figure. The present analysis leaves a number of questions unanswered which logically might have been included. The effect of community development upon land values for instance is a most important consideration but available data have not yet given the means to measure the effect of the general character of the community upon land prices.

Sources of Data.—Sales prices of farms may be secured from county offices where they are legally on record, from railroad companies, from banks, from farmers, and other agencies which have compiled sales prices from one of the above sources. The Federal Land Bank has within its files more than 30,000 cases where applications for loans have been made in which the applicant has declared the purchase price paid for his farm. More than 18,000 of these are in California, 9,000 in Utah, 700 in Nevada and 3,000 in Arizona. A large part of the records on file in the bank are for farms covered by

active federal farm loans. In 1926, 854 loans were closed in California, 295 in Utah, 54 in Nevada, and 143 in Arizona. A little more than half the total are rejects where loans have not been approved or are cancellations of loans which have been paid up. Nearly 5,000 of these farms have changed hands since the loans have been in the bank. The sale of a farm on which a federal farm loan has been granted is called a resale. These resale prices, declared before a notary by the purchaser, are recorded in the bank at the time the transfer is made. In 1926, 261 resales were made in the Eleventh Federal Farm Loan District. Of these, 146 were in California.

This source of sales price data is especially interesting because of the fact that a more or less complete description of each farm accompanies the record of the sale price. This information is more complete for the later years because appraisal methods have gradually improved and basic information has been collected, making possible more reliable information concerning conditions throughout the Farm Loan District. While there are many limitations to the use of these data for research, because they were collected for another purpose, they furnish the basis for some very interesting analyses of the relation of sales prices of land to such factors as affect selling prices. These data form the principal basis of the study. While most of the available prices have been used in time series, only a small number of the total number of cases enter into the detailed correlations because of eliminations described later.

Data in Appraisers' Reports and Applications for Loans.— Appraisers' reports and applications for loans contain, among other information, a record of the purchase price of the land, estimates of gross income, net farm income, and a financial statement of the farmer. The utilization of the farm is given, showing the acreages of the different crops; and in the application blank, the yields of the important crops for the year the application was made are itemized, while in the appraiser's report the appraiser's estimate of the average yield is recorded. Notes as to topography are included, and the soil type is given by the appraiser where there has been a soil survey, or described in more or less detail where there has been no soil survey. The exact location of the farm is recorded and its distance from railroad station, church, school, and state roads. In later years, very adequate description of drainage and irrigation factors have been attached to the appraiser's report on a special blank. Information is available concerning costs of irrigation, including bonded indebtedness, interest on bonded debt, and operation and maintenance costs.

Where pumps are installed for irrigation, information is usually given to make possible an estimate of the cost of operatioin. Information is also included, though not always complete, concerning the value of improvements made on the farm between the time of purchase and the time of appraisal. Appraised values of land and buildings are recorded separately.

Purchase Price and Resales Price Data.—Purchase price and resales price data are subject to certain inaccuracies. It was expected that purchase price data would be subject to a bias due to the desire on the part of some applicants to secure as large a loan as possible. On the whole, however, it is believed that the applicants have been honest in their statements. Purchase price has the disadvantage of containing in many cases the price paid for stock, equipment, etc., which had not been mentioned in the applicant's report. Usually these cases can be detected and eliminated. Data on purchase price are also given by the applicant sometimes years after the transfer occurred, thus making the memory of the farmer giving the report a source of error. The purchase price as recorded applies, in each case, to a time prior to the time of application and appraisal. If this is several years, the descriptive matter concerning the farm, recorded by the applicant and the appraiser at the time of appraisal, applies to the farm some time later than the date of purchase. In the meantime, improvements may have been made and the descriptive information may have little significance in the analysis of the factors affecting purchase price. The resale price is of record usually some years later than the date at which description is available. No record of improvements during this intervening time is given. Studies of the effect of this lapse of time have been made by examining average sales price of farms by groups of different lengths of time between appraisal and resale. There seems to be little change where the farm is in a fair degree of development at the time of appraisal. By including in correlations of factors affecting sales price only such farms as are in a fair state of development and farms sold not more than two years subsequent to appraisal, error from this source is greatly reduced.

Income Data.—Estimates of net farm income and gross income are probably of very little value. The statement of the applicant is often very different from that of the appraiser in respect to income. Accurate estimates of income are extremely difficult to make and discrepancies are expected in such data.

Dynamic Economic Factors and Their Significance with Relation to Land Price

Land prices are subject to both secular and cyclical changes. The relationship between industrial conditions, agricultural prices, and land prices is not a simple one. The effect of changes in demand for land, on cyclical deviations in land prices, depends upon two sets of variables. One of these is a composite of industrial activity, and the other pertains directly to agricultural income. These two influences upon demand for land and a group of factors which influence economic supply of land determine the general land price level.
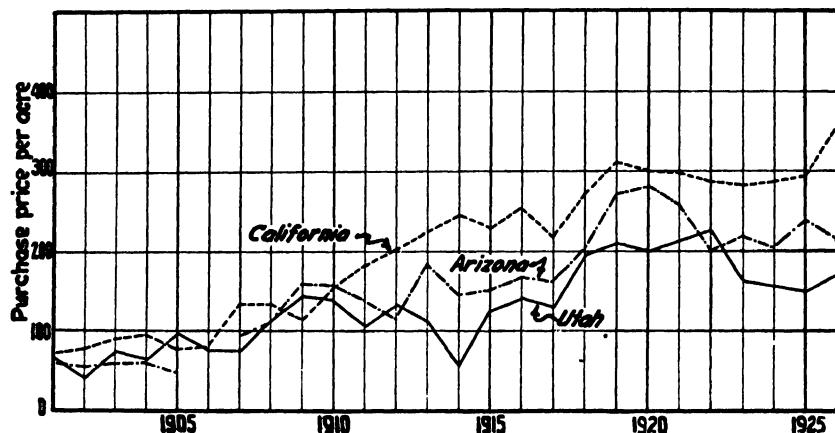
Fig. 1. Average purchase prices of land covered by Federal farm loans in California, Arizona, and Utah, 1901–1926.

Trends in Purchase Price of Farms Covered by Federal Farm Loans.—Purchase price of farms covered by Federal Farm Loans is useful within certain important limitations in studying the secular trend of land prices over a period of years. Time series of land price for different types of agriculture have important differences. Combining in a single land price series prices of farms of widely varying sizes, different types of agriculture, and varying improvement values is sure to result in misuse and wrong interpretation by the majority of those interested, if care is not taken in weighting the series according to the purpose intended.

Average annual purchase price of farm land in Arizona, Utah and California are shown in figure 1 and in tables 1 and 2. Figure 2 and table 2 show the average purchase prices of farm land covered by

federal farm loans in California from 1901 to 1920 inclusive. Values of improved farms and unimproved farms published by the U. S. Department of Agriculture[9] from 1912–1926 inclusive are also shown. In California, the purchase prices have been sorted according to type of agriculture. The groups for individual crops contain so few cases in certain years that time series were impracticable. When combined into the general groups of permanent crop and field crops, some interesting comparisons have been made possible. Since 1918, purchase prices of permanent crop land in California have averaged about $400 per acre, while the purchase price of field crop and dairy land taken together has averaged about $230 per acre. Permanent crops and field
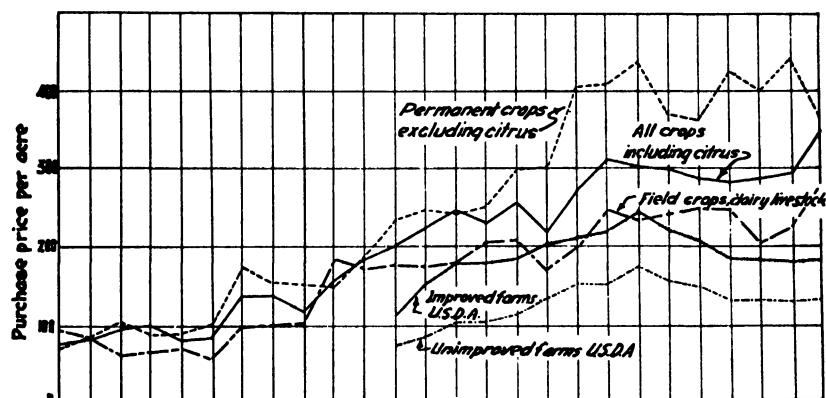
Fig. 2.—Average prices of land covered by Federal farm loans in California compared with prices of farm land compiled by the U. S. Department of Agriculture. The data are shown in table 2.

crops combined in the all-crop land price series have averaged a little less than $300 per acre. Prior to 1918, there was, as is well known through other land value studies, a gradual rise in land values, being most rapid during the period shown by these land price series. It must be borne in mind that these prices apply to land purchased prior to application for loans in the bank, and in a large number of cases include prices paid for practically undeveloped land. In this respect they differ materially from lands transferred since loans have been issued by the bank. The latter which have already been referred to as resale prices will be considered after certain characteristics of average purchase prices for the different years have been described.

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[9] Bureau of Agricultural Economics, Prices of farm products received by producers. 4. Mountain and Pacific States. U. S. D. A. Stat. Bul. 17:152, table 77. Government Printing Office, Washington, D. C.

In making comparisons between the U. S. Department of Agriculture index for improved farms and unimproved farms in relation to the purchase prices of land covered by Federal Farm Loans, it will be noticed that the U. S. Department of Agriculture index is much lower than that of this research. An important cause for this difference is probably in the manner of computing the average prices repre-

TABLE 1

AVERAGE PURCHASE PRICE PER ACRE OF LAND COVERED BY FEDERAL FARM LOANS IN ARIZONA AND UTAH, 1901-1926

Year	Arizona		Utah	
	Frequency	Average price	Frequency	Average price
		Dollars		*Dollars*
1900	5	49	7	115
1901	2	62	5	68
1902	3	57	8	42
1903	5	67	10	76
1904	4	65	8	64
1905	2	50	15	99
1906	....	......	15	77
1907	7	94	8	76
1908	3	107	12	115
1909	11	158	11	144
1910	7	157	15	140
1911	11	139	13	106
1912	22	117	20	133
1913	10	184	17	188
1914	14	146	15	58
1915	15	152	14	125
1916	31	170	36	141
1917	77	163	37	130
1918	57	202	52	196
1919	54	275	63	209
1920	63	284	63	200
1921	37	261	22	214
1922	32	203	19	226
1923	47	218	28	161
1924	21	205	34	154
1925	9	239	18	148
1926	4	214	14	171

sented. The usual custom in computing such an index is to calculate average prices by dividing total value by total area. Such an index is not representative of prices that the majority of farmers paid for their farms nor would it be representative of that area where greatest values are concentrated. The average is unduly weighted by the larger farms. The purchase price averages used in this study have been

computed by adding together the average prices per acre for all of the farms in the sample for a given year and dividing by the total number of cases included. By this method more weight is given to the price per acre of that type of farm of most common frequency rather

TABLE 2

AVERAGE PURCHASE PRICE OF LAND COVERED BY FEDERAL FARM LOANS IN
CALIFORNIA FOR THE PERIOD 1901–1926, INCLUSIVE, COMPARED WITH
THE FARM LAND PRICE SERIES FOR CALIFORNIA COMPILED BY
THE U. S. DEPARTMENT OF AGRICULTURE, 1912–1926

Date	Average purchase price per acre of land covered by federal farm loans			U. S. D. A.* farm land value	
	All crops, including citrus	Permanent crops, excluding citrus	Field crops, dairy, livestock	Improved farms	Unimproved farms
	Dollars	*Dollars*	*Dollars*	*Dollars*	*Dollars*
1901	72	68	91	.. .	. .
1902	79	89	81	......	......
1903	91	102	59	. .	.... .
1904	96	84	63	.... .	. .....
1905	77	85	68	... .	. ..
1906	82	99	55	.... .	. . .
1907	135	172	93	. . . .	... .
1908	134	152	98	. .....	.... .
1909	114	149	98	......	. . . .
1910	155	145	138	. ..	. ..
1911	183	186	131	... .	......
1912	201	233	175	107	70
1913	225	245	173	150	85
1914	245	242	179	175	100
1915	229	250	205	175	100
1916	253	298	208	180	110
1917	218	300	170	200	130
1918	272	406	195	207	148
1919	310	408	246	218	150
1920	300	439	230	240	170
1921	298	369	241	218	155
1922	286	363	248	206	146
1923	281	425	247	182	129
1924	286	401	205	180	128
1925	293	443	223	178	126
1926	356	368	278	180	130

* Bureau of Agricultural Economics. Prices of farm products received by producers. Part 4. Mountain and Pacific states. U. S. D. A. Stat. Bul. 17: 152. Washington, D. C., March, 1927.

than to total acreage. More weight is given to small farms where larger values are concentrated inasmuch as they are the most frequent sizes. More weight is given to that type of farm that the "average" farmer is thinking of buying or selling. A special study has been

made to determine the difference between a time series derived by this method of frequency weighting and time series derived by two other methods in common use. The same data are used in the three types of averages, but one series is weighted by value, another series by acreage, while a third is in effect weighted according to the type of farm of greatest frequency. The average price per acre for each farm was computed by dividing its value by the number of acres in the individual farm. The averages for the year were computed by dividing the sum of the average prices per acre by the total number of farms in the group. As was expected, and in conformity with the variance between the U. S. Department of Agriculture index and the purchase price series of this analysis, the series weighted by acreage is much below that of the other two, while that weighted by value gives a series higher than that given by the other two methods. The results of these computations are shown in figure 3. The purchase price index weighted by the frequency is not representative of the average price per acre which was paid for large farms in the years indicated. In a state where land prices vary from a dollar or two an acre to thousands of dollars per acre and where sizes of farms vary from thousands of acres to a fraction of an acre, there is a question as to the real significance of any form of series of land prices which does not represent a specific class of farm. It seems that the one representing the farm of most frequent size would be more representative than the one giving weight more particularly to large areas of low-priced land.

It must be remembered that in addition to the method of computing averages, there may be another reason for the difference in the purchase price index and the U. S. Department of Agriculture series. The purchase price index is for farms which have for the most part been selected as suitable for federal farm loans. This is probably not an important cause of the difference. In many cases, however, the purchase price included in the average was for land which at the time of purchase would not have been considered sufficient security for the loan and that the development since purchase, in many cases, undoubtedly has been the basis of the security. Improved farm land values in California from 1918 to 1927, according to the U. S. Department of Agriculture index, have averaged $201 an acre. Land values during the earlier portion of this period were much higher than during the later years. From 1918 to 1922 inclusive the average value was $218 an acre while from 1923 to 1927 inclusive the average was $180 an acre.

Another source of misinterpretation of such series may arise from the fact that more small farms come into the average as time goes on. Census data and the records of the Federal Land Bank both show a gradual diminution in the size of farms and since small farms sell for higher prices per acre than large farms for many reasons, a time series of land prices which does not give consideration to size changes is subject to an exaggeration of an upward trend in land price.
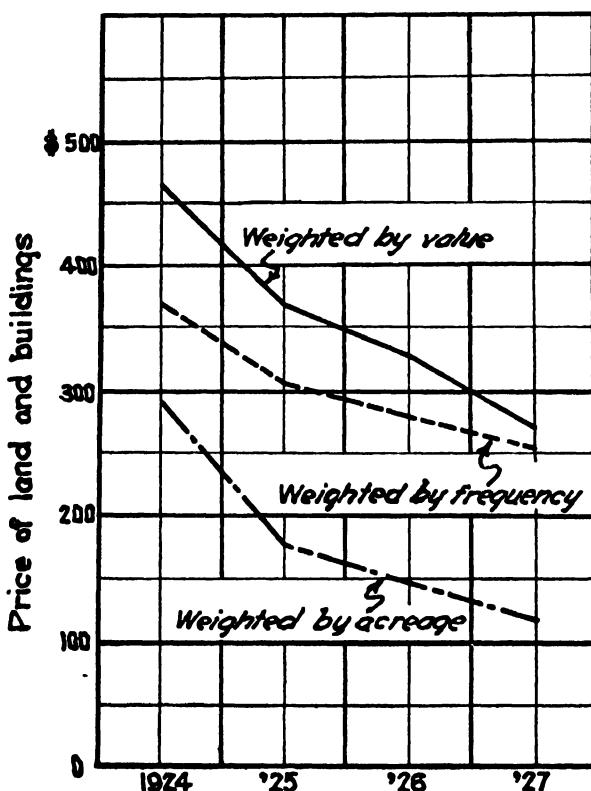

Fig. 3. The effect of methods of weighting upon time series of land prices.

When the purchase price series is compared with the resale price series, discussed later, it will be seen that the peak of land prices occurred at an earlier date in the former series. There is no doubt that such a difference actually occurred in the case of farms resold after the issuance of a loan because of a greater degree of development. There may have been, however, certain erratic discrepancies in each of the series in 1921 and 1922 when such small numbers of farms changed hands as to cause samples of comparatively small numbers of

cases. Inasmuch as the resale prices are for more highly developed farms and farms which were probably in the process of development at the time deflation began, there is reason to believe that the increase in development resulted in a continuation of rising values due to added improvements. In the case of purchase prices, however, which in large numbers of cases were the sales of land in development projects, the slump was felt sooner.

The decline in purchase prices in 1917 is not shown in the Department of Agriculture series. This decline is probably significant for it occurred in several independent series. It is present in the California permanent crop land series, in the California field crop and dairy farm land series, and in the all-crop lands of Utah and Arizona. It is probably explained by the declining demand for land by mobilization of troops, and increased industrial activity.

Later discussions of the effect of size and other variables upon land price indicate the inadequacy of ordinary time series of land prices for most purposes. In fact they may be actually misleading. If a time series must be used for deflation purposes, relatives will serve that purpose. Such a series is not so susceptible to the effects of poor weighting.

Cyclical Analysis of Resale Prices of Farm Land.—There is a direct relationship between cycles in the price of improved land in the San Joaquin Valley, California, since 1921 and cycles of industrial and financial activities. The correlation between land price and daily pig iron production in the United States is a fair example of this relationship. Figure 4 and table 3 show monthly average resale prices per acre of land covered by federal farm loans in the San Joaquin Valley, California, which changed hands each month from September, 1918, until March, 1927. Because of a comparatively small number of cases in each month, there is considerable variation in these monthly averages. In order to smooth out these irregularities and to study the cyclical changes in land prices, twice iterated three-months moving averages have been applied to the raw monthly averages. The inflection points of the resulting series have been connected in smooth curves which we will call, following Frisch,[10] the originator of this method of time series analysis, the first trend. The first trend shows cyclical variations, with seasonal and erratic fluctuations eliminated.

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[10] Dr. Ragnar Frisch, Lecturer of Economics and Mathematical Statistics in the University of Oslo, Norway, has through personal interview described the essentials of his method to the writer.
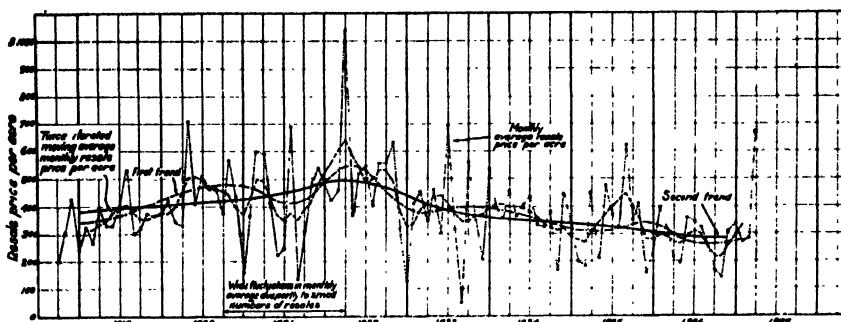
Fig. 4.   Analysis of cyclical variation in resale prices of land covered by Federal farm loans in the San Joaquin Valley, California.
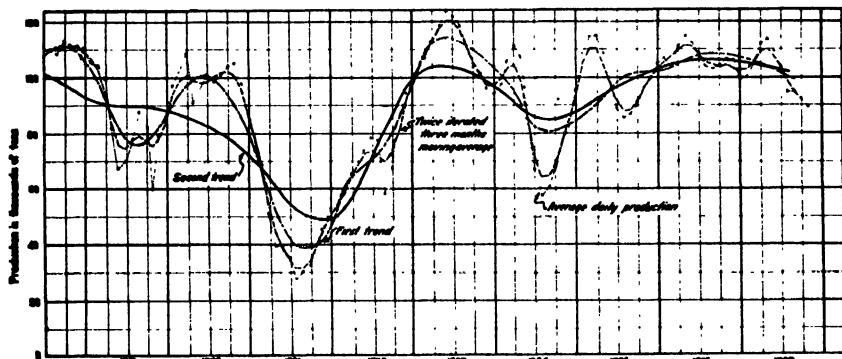

Fig. 5.   Cyclical variations in average daily pig iron production in the United States, 1918–1927.
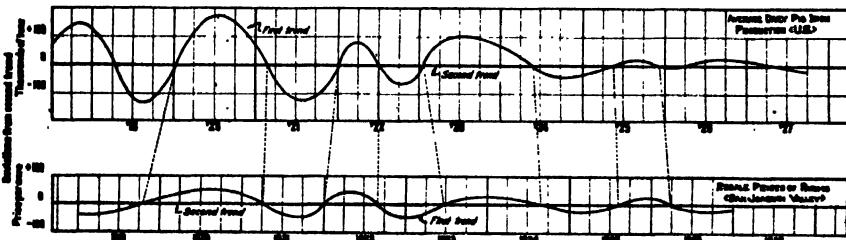

Fig. 6.   Comparison of cyclical variations in pig iron production in the United States, and resale prices of farms covered by Federal farm loans in the San Joaquin Valley, California.

TABLE 3

AVERAGE MONTHLY SALES PRICES OF LAND COVERED BY FEDERAL FARM LOANS
IN SAN JOAQUIN AND SACRAMENTO VALLEYS, CALIFORNIA, AND
IN THE STATES OF UTAH AND ARIZONA

Year	Month	San Joaquin		Sacramento		Utah		Arizona	
		Fre-quency	Price	Fre-quency	Price	Fre-quency	Price	Fre-quency	Price
			Dollars		*Dollars*		*Dollars*		*Dollars*
1918	January	1	264						
	February								
	March					1	46		
	April								
	May								
	June			1	20				
	July	1	211	1	700				
	August								
	September	1	200	1	113	1	487		
	October					4	133	1	240
	November	1	425						
	December	2	238	1	187	2	190	2	75
1919	January	1	325			5	203	1	55
	February	4	265	1	187	1	31	1	44
	March	3	395	3	120	7	138	3	242
	April	5	327	1	89	9	80	2	102
	May	6	327	2	352	5	126		
	June	3	400	3	113	2	58	1	141
	July	7	527	3	75	4	263		
	August	11	299			4	94	2	130
	September	9	315			3	110		
	October	9	370	5	205	5	127		
	November	10	361	4	283	9	185		
	December	15	371	9	222	6	153	4	205
1920	January	19	418			8	236	3	181
	February	19	341	5	136	14	197		
	March	10	327	7	298	14	197	2	306
	April	9	702	2	142	3	199	3	188
	May	9	403	1	11	9	165	1	284
	June	9	504	2	65	3	122	3	41
	July	4	465	3	350	4	203		
	August	7	457			6	127	2	26
	September	2	369			4	284	3	438
	October	7	561	6	200	3	215	1	473
	November	4	421	3	451	4	144	1	750
	December	2	154	2	182	2	103	1	200
1921	January	8	360			4	140		
	February	4	591	2	145	5	142	1	181
	March	3	585	2	223	3	199	2	107
	April	4	432	1	19	8	96	3	98
	May	3	220	2	138	4	187	1	26
	June	4	246			2	182		
	July	3	683						
	August	1	134	1	255				
	September			2	217	1	103		
	October	5	468	1	37	3	170		
	November	8	535	1	141	13	77		
	December			2	212	6	142	1	75

TABLE 3 (continued)

Year	Month	San Joaquin		Sacramento		Utah		Arizona	
		Frequency	Price	Frequency	Price	Frequency	Price	Frequency	Price
			Dollars		*Dollars*		*Dollars*		*Dollars*
1922	January.......	4	416	1	400	5	140	3	86
	February.... ...	4	455	1	300	4	188	4	194
	March........ ..	2	1128	1	280	5	145	2	159
	April	4	364	1	26	5	215	2	168
	May........... .	5	513	..	... .	9	149	3	240
	June	5	540	1	580	6	151	1	94
	July................	4	401	. ...	. ...	2	247	....	.. . .
	August.........	5	553	1	26	2	168	2	88
	September	2	551	5	331	9	182	2	149
	October	4	627	. ...	.... ..	7	121	4	161
	November .	1	315	1	423	14	111	2	178
	December. ..	4	126	3	158	5	113	2	231
1923	January	7	406	2	174	9	192	2	192
	February	3	455	2	339	7	51	2	211
	March........ ..	4	341	3	216	11	113	4	102
	April...... . .	3	455	3	248	6	152	4	210
	May................	5	299	2	197	10	111	1	250
	June	2	688	1	12	4	106	.. . .. .	. . . .
	July......	5	315	2	370	3	214	1	95
	August	1	52	1	533	4	106	3	39
	September...	3	556	3	155	4	113	1	188
	October	1	347	2	220	5	209	3	179
	November	5	202	5	219	12	156	2	206
	December ..	6	532	1	200	11	118	2	244
1924	January..... .	9	392	. .. .	.. . .. .	14	132	8	209
	February .. .	8	254	2	312	14	152	1	275
	March........ ..	8	454	6	286	12	186	4	202
	April................	4	359	3	540	11	154	3	182
	May	6	402	.. ..	....	4	103	1	62
	June	7	434	2	538	5	129	3	138
	July..........	5	334	4	644	4	126	1	148
	August......... .	5	323	2	295	5	129	2	134
	September ...	6	374	2	151	4	118	1	213
	October	6	162	2	374	5	150	....	.. . ..
	November ..	7	441	. ...	....	2	132	5	145
	December......	7	324	2	95	6	119	5	226
1925	January..........	8	198	3	209	4	94	8	200
	February........	7	180	2	255	7	86	3	181
	March.............	8	448	2	397	13	107	3	181
	April................	7	208	2	348	4	97	1	116
	May...........	7	468	1	318	5	127	1	300
	June	5	370	3	204	5	92	2	226
	July................	9	314	2	100	2	158	2	194
	August............	3	617	3	386	3	135	1	62
	September...	7	347	2	158	4	29	5	124
	October	5	408	3	468	5	153	4	213
	November ..	6	153	1	234	8	104	1	191
	December......	7	238	2	422	11	170	6	118

TABLE 3 (continued)

Year	Month	San Joaquin		Sacramento		Utah		Arizona	
		Fre-quency	Price	Fre-quency	Price	Fre-quency	Price	Fre-quency	Price
			Dollars		*Dollars*		*Dollars*		*Dollars*
1926	January.............	8	389	2	358	12	127	7	235
	February........	8	315	4	184	6	130	3	175
	March............	9	214	3	356	17	169	3	262
	April.............	11	189	3	398	8	102	2	184
	May.............	10	354	3	99	5	61	2	98
	June	6	340	2	248	3	42	2	24
	July...	4	320	3	241	3	88	1	185
	August... ..	8	254			5	92	1	372
	September	3	167	3	248	7	99	3	199
	October	4	138	2	461	4	151	7	254
	November	4	304	4	233	7	175		
	December...	5	332	3	283	10	67	3	171
1927	January ...	16	269						
	February	16	266						
	March ...	13	307						
	April	17	240						
	May	23	425						
	June	12	209						
	July..	6	220						
	August... .	12	250						
	September	17	293						
	October ...	16	209						
	November	9	204						
	December ..	9	238						

Seasonal variations in land price indicate that high-land prices tend to occur in the second quarter of the year, that is, from March to June. This, of course, is coincident with high seasonal demand and indicates that most farm land is exchanged at a time which gives an advantage to the seller. The inflection points of this first trend have been used in constructing a second trend. This second trend follows the general long-time tendency of land prices. The method is treated more fully in the discussion of "The Adequacy of the Frisch Method of Time Series Analysis," found in the section "Statistical Analysis" at the close of this bulletin. At present, the economic and not the statistical aspects of the problem are under consideration.

Figure 5 shows graphically the average daily pig iron production in the United States by months from 1918 to 1927. The Frisch method of analysis has been used as in the case of the land prices. The regional monthly averages were treated with a twice iterated three-months moving average. The inflection points of the resulting series were connected to form the first trend. The second trend is the line

passing through the inflection points of the first trend. Deviations of the first trend from the second trend give us the cyclical variations which are comparable to the cycles of the same order in land prices.

Figure 6 shows the deviations of the first trends of each of these series from the second trends, land price being lagged two months behind pig iron production. While there is only a fair correlation, and the series cover only comparatively short periods of time in each case, there is considerable evidence of direct relationship between the two series. The lag of land prices behind pig iron production indicates that industrial activity is followed soon by corresponding changes in the price of improved high-priced land. That the relationship between industrial conditions and agricultural prices is not a simple one is emphasized by the occurrence of important exceptions to the direct relationship. As will be seen later, the demand for undeveloped low-priced land follows a different cyclical tendency. Resale prices are for land which is in a fairly complete state of development. Loans are not awarded where farms are unimproved.

In the discussion of purchase prices, certain precautions were found to be necessary in the use of time series of land prices. The same precautions should be followed in the use of time series based upon resale prices. Although the prices are more accurate, the series are subject to changes in the proportion of farms of different sizes.

If a series is a composite of land prices in different localities, not only may cyclical tendencies be different, but the long-time trends in the different regions may follow along entirely different lines. As a matter of fact, cyclical changes in the different regions are more nearly alike than are the trends. Figure 7 shows recent trends in resale prices of land in different sections of the Eleventh Federal Farm Loan District. Cyclical tendencies are similar but trends are not. Farm land prices rose higher in the San Joaquin Valley than in other parts of the district and retained their high level until 1922. Since 1922 the trend has been downward. For the rest of the State of California, the trend was similar to the San Joaquin Valley until the later part of 1923 when a decline set in after which there have been some signs of recovery. In the Sacramento Valley, excluding those speculative sections where the Federal Land Bank has not made a practice of extending credit, land prices followed a steadily increasing trend until late in the year 1924, after which there was a slight decline. In Arizona and Utah the high points came much earlier. The peak of land prices occurred in Arizona in 1920, while in Utah the higher values occurred earlier in 1919. Since that time, there has been a very

slight decline in the general trend. These regional differences in land
price trends give a feeling of insecurity in the use of a general price
series for following land value changes in local areas. Even the trend
for the San Joaquin Valley should be applied to local areas within the
San Joaquin Valley with caution. General crop areas are likely to have
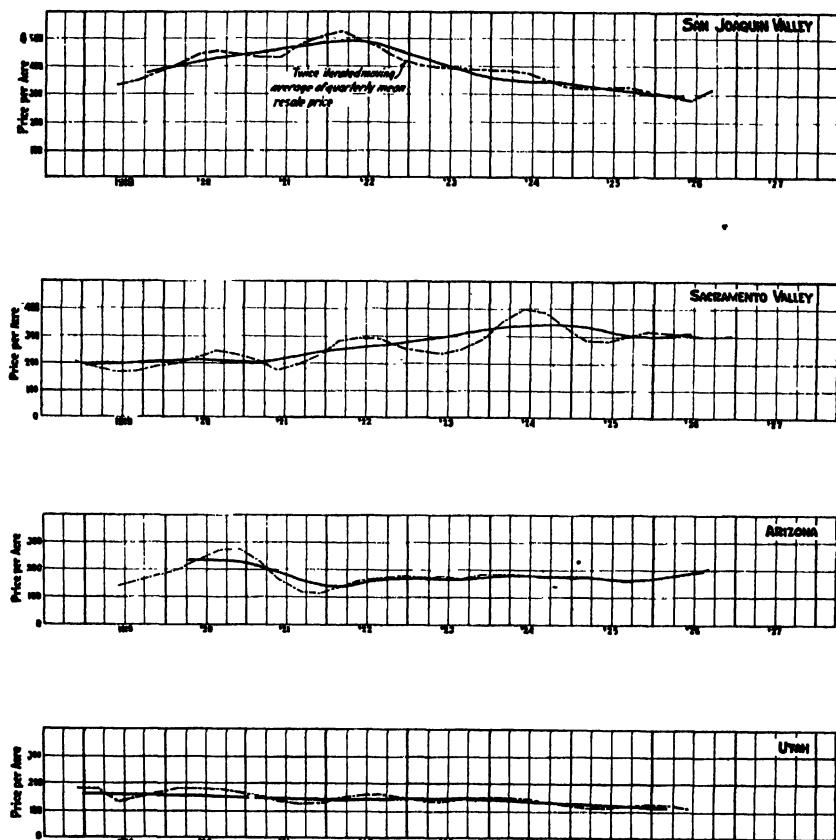
Fig. 7. Recent trends of resale prices of land in different sections of the
Eleventh Federal Farm Loan District.

different land price trends from those of permanent crop lands. There
is some indication, though not a persistent tendency, that cycles in
purchase price of dairy and general crop farms are inverse to cycles
in prices of permanent crop lands. The actual process of land price
deflation is difficult and so far satisfactory methods are not available.

A more detailed analysis was made of some of the different counties
in the San Joaquin Valley. Merced and Fresno counties seemed to
follow similar land price changes, the difference being in the height

of the price level. These county series are not shown graphically
because of insufficient data to make them continuous. Sufficient data
have.not been available to create land price trends for small areas.
Such trends would be necessary if an accurate determination were to
be made of local changes in land value. Even if a time series for a
local area were available, in light of what we have said about prices
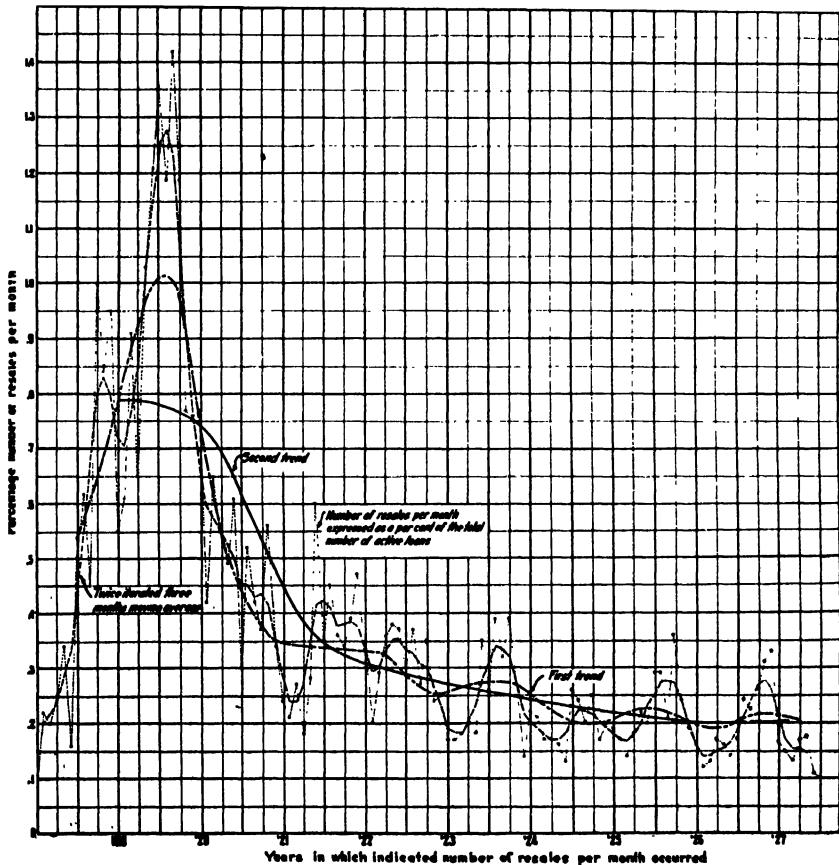
Fig. 8. Analysis of cyclical variation in the number of resales per month,
expressed as a per cent of the total number of loans in the Eleventh Federal
Farm Loan District.

of land utilized for different crops, prices of all farm lands would not
necessarily follow this general trend. We must depend therefore on
time series for larger areas and study the causes for regional differ-
ences. In this way corrections may be made on the basis of local
characteristics. Dynamic factors must take their place with other
variables in the complex process of "unscrambling" the interacting
effects of the many elements affecting land price.

Factors Influencing Demand for Farm Land. — Demand for improved farms of high per-acre value seems to have different causes from those which produce the fluctuations in the demand for raw, undeveloped low-priced land. The number of resales per month among the farms covered by federal farm loans gives us an index of the rate of transfer with respect to the improved farms involving higher acreage values. Figure 8 and table 4 show graphically the rate at which farms in the Federal Land Bank changed ownership during the years 1918–1927 inclusive. The number of resales per month expressed as a per cent of the total number of loans in the Eleventh Federal Farm Loan District declined rapidly, with minor variations, from early in 1920 to 1921. Since 1921, there has been a further gradual decline up to the year 1927. There is some indication of a flattening out of the trend in the later post-war years. During the month of February, 1920, a little more than 1.4 per cent of the farms on record in the Federal Land Bank of Berkeley changed hands. In February, 1927, about 0.23 per cent of the total number of farms on record in the bank changed hands.

The declining trend in rate of transfer is accompanied by a decline in trend in the selling price of these same farms. There is no definite proof that there is any causal relationship between the decline in the trends of the price of land and rate of transfer. The decline in rate of transfer could be due to diminished demand for land by purchasers either because of decreased prospects of adequate returns or because of greater opportunity offered in other fields. The decline in rate of transfer could be due to the reluctance of the owners to part with their farms at available prices offered. There is probably no real difference in the fundamental causes for the decline in the rate of transfer whether it acts through its effect upon the willingness of the owners to sell or upon the demand by purchasers. When we consider short time fluctuations, however, we find a persistent inverse relationship between land prices in any year and the rate at which land sales take place the next. Figure 9 shows the relationship of prices of farm land to the number of sales per month with the resale transfer curve lagged one year. This chart is constructed from the first and second trends of resale prices shown previously in figure 4, and the cycles of rate of transfer of the same order taken from figure 8, the second trend in each case being represented by the line 00. The inverse relationship between land prices and rate of transfer seems to be more significant and the correlation seems to be higher than any relationship which may be found to exist between the cyclical variations in rate of land

transfer and business conditions or wholesale prices. Although there is some indication of such a relationship, it is probably due to the indirect effect of industrial or financial conditions on rate of transfer through its effect upon land price.

A digression from the discussion of the results of the analysis of transfers of farms on file in the Federal Land Bank is necessary at this point to complete our understanding of the factors influencing demand for land. For the study of a clear-cut case of demand for undeveloped land, where the price of the land is not a retarding factor, operations under the Homestead Act give us the desired data.
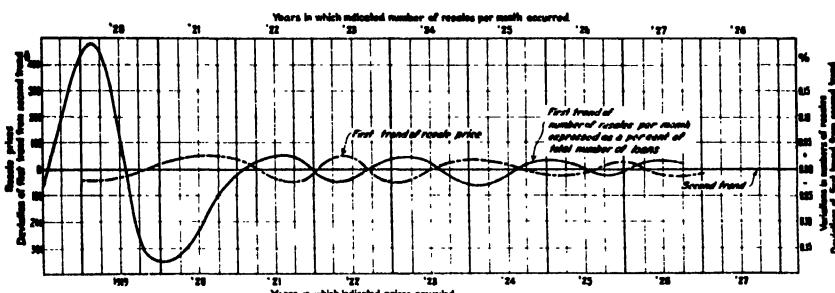
Fig. 9.   Relation of prices of farm land to numbers of sales per month the following year. This figure is derived from figures 4 and 8.

In considering the conclusions drawn, however, it must be remembered that many economic changes have occurred since the years covered by this series and that the immigration laws of the country may affect the tendencies observed. When homestead entries are analyzed from the standpoint of cyclical variations, the conclusion is that the demand for raw, low-priced land is much more subject to industrial conditions than is the case with higher priced developed farms. In the first instance, increased activity under the Homestead Law indicates a definite movement to the land by those who make permanent settlers. In the case of the rate of transfer of improved lands, each purchaser moving to his newly purchased farm displaces another farmer so that the movement is not one which is all in one direction. There is a movement away as well as a movement to the land. The farmer displaced by the purchaser may himself become a purchaser. Furthermore, the purchase of improved farms involves the expenditure of considerable amounts of capital by the purchaser even where extensive credit is given. The improved farm, therefore, is an outlet for a different class of labor from the unimproved farm which attracts large numbers of those formerly employed in industry seeking new employment when industry fails them.

TABLE 4

NUMBER OF RESALE TRANSFERS PER MONTH EXPRESSED AS A PER CENT OF TOTAL NUMBER OF LOANS IN THE ELEVENTH FEDERAL FARM LOAN DISTRICT

Year	Month	Number of resales	Number of active loans in bank*	Per cent of resales to number of loans	Year	Month	Number of resales	Number of active loans in bank*	Per cent of resales to number of loans
1918	Jan	1	630	.16	1922	Jan	22	4,930	.45
	Feb	...	...	...		Feb	18	5,050	.36
	Mar	5	850	.59		March	18	5,240	.34
	April	3	960	31		April	21	5,400	.39
	May	3	1,090	.28		May	26	5,550	.47
	June	1	1,230	.08		June	19	5,750	.33
	July	3	1,350	.22		July	12	5,900	.20
	Aug	3	1,480	.20		Aug	18	6,160	.29
	Sept	4	1,600	.25		Sept	23	6,450	.36
	Oct	6	1,750	.34		Oct	26	6,750	.38
	Nov	3	1,860	.16		Nov	26	7,050	.37
	Dec	10	2,000	.50		Dec	22	7,330	.30
1919	Jan	13	2,120	.62	1923	Jan	28	7,650	.37
	Feb	10	2,250	.45		Feb	21	7,920	.27
	March	24	2,400	1 00		March	29	8,200	.35
	April	21	2,500	.84		April	20	8,380	.24
	May	25	2,650	.95		May	21	8,560	.25
	June	15	2,750	.55		June	15	8,700	.17
	July	17	2,860	.61		July	15	8,850	.17
	Aug	27	2,980	.91		Aug	16	8,950	.18
	Sept	20	3,090	65		Sept	18	9,020	.20
	Oct	33	3,200	1 03		Oct	16	9,150	.18
	Nov	40	3,300	1 21		Nov	32	9,250	.35
	Dec	46	3,370	1 36		Dec	24	9,350	.26
1920	Jan	41	3,450	1.19	1924	Jan	37	9,450	.39
	Feb	50	3,520	1.42		Feb	31	9,550	.32
	March	43	3,600	1 19		March	38	9,650	.39
	April	28	3,650	.77		April	26	9,750	.27
	May	28	3,700	.76		May	14	9,840	.14
	June	29	3,750	.77		June	22	9,930	.22
	July	16	3,800	.42		July	21	10,030	.21
	Aug	25	3,830	.65		Aug	17	10,130	.17
	Sept	21	3,850	.55		Sept	17	10,230	.17
	Oct	19	3,900	.49		Oct	17	10,300	.16
	Nov	24	3,920	.61		Nov	14	10,400	.13
	Dec	12	3,950	.30		Dec	27	10,500	.26
1921	Jan	21	4,000	.52	1925	Jan	25	10,560	.24
	Feb	17	4,040	.42		Feb	21	10,650	.20
	March	15	4,080	.37		March	27	10,750	.25
	April	23	4,110	.56		April	18	10,800	.17
	May	14	4,170	.34		May	22	10,900	.20
	June	10	4,250	.24		June	21	11,000	.19
	July	9	4,320	.21		July	19	11,080	.17
	Aug	12	4,400	.27		Aug	15	11,150	.14
	Sept	8	4,500	.18		Sept	23	11,250	.18
	Oct	13	4,580	.28		Oct	25	11,300	.22
	Nov	28	4,700	.60		Nov	23	11,400	.20
	Dec	17	4,800	.35		Dec	33	11,500	.29

TABLE 4 (continued)

Year	Month	Number of resales	Number of active loans in bank*	Per cent of resales to number of loans	Year	Month	Number of resales	Number of active loans in bank*	Per cent of resales to number of loans
1926	Jan............	34	11,600	.29	1927	Jan.........	31	12,840	.24
	Feb...........	24	11,700	.21		Feb........	29	12,940	.22
	March......	42	11,790	.36		March...	34	13,050	.26
	April........	29	11,880	.24		April....	41	13,170	.31
	May.........	23	11,950	.19		May.......	44	13,300	.33
	June	19	12,070	.16		June	22	13,400	.16
	July.........	15	12,160	.12		July.......	20	13,500	.15
	Aug..........	16	12,260	.13		Aug..	18	13,600	.13
	Sept.........	21	12,400	.17		Sept..... .	23	13,700	.17
	Oct...........	20	12,500	.16		Oct.........	24	13,800	.17
	Nov.........	18	12,600	.14		Nov..	15	13,900	.11
	Dec..........	21	12,750	.17		Dec..	14	14,000	.10

* Number of active loans in the bank for any month were estimated by reading values from a curve connecting the annual totals. While they are sufficiently accurate for the purpose for which they were used here they are not the exact numbers of active loans.

In a previous bulletin,[11] it has been shown that there is an inverse relationship between the all-commodities wholesale price index and final homestead entries lagged for the period between original and final entry. This tendency has been studied further in the present investigation. Effective demand for undeveloped land, where land price is not a dominant consideration, varies inversely with wholesale prices of all commodities. Figure 10 shows original and final homestead entries plotted for the years 1863 to 1923 inclusive.[12] The final entry graph is lagged for the period of three or five years required for proof. Many original entries did not become final entries. The final entries are the ones, therefore, which show effective demand for land. The percentage of original entries which became final, that is, the effective demand for land, is correlated with business conditions at time of entry, business conditions during the early period of settle-

[11] Weeks, David and Charles H. West. The problem of securing closer relationship between agricultural development and irrigation construction. California Agr. Exp. Bul. 435:99 p. 1927.

[12] Source of data:

 1863–1880. Report of Public Land Commission.   p. 351–355.   1881.
 1881–1882. Report of Public Land Commission.   p. 1016.   1884.
 1882–1883. Public Domain.   Report of Public Land Commission.   p. 1284.
  1884.
 1884–1899. Report of Commission of General Lands.
 1900–1906. Congressional Records.   1899—p. 3914.   1900—p. 4100.   1901—
  p. 4289.   1902—p. 4457.   1903—p. 4644.   1904—p. 4797.   1905—
  p. 4958.   1906—p. 5117.
 1907–1926. Report of Commission of General Land Office in Dept. of
  Interior Reports.

ment, and with the total number of entries. Figure 11[13] is a cyclical analysis of wholesale prices and forms the basis together with figure 10 for figure 12 which shows graphicall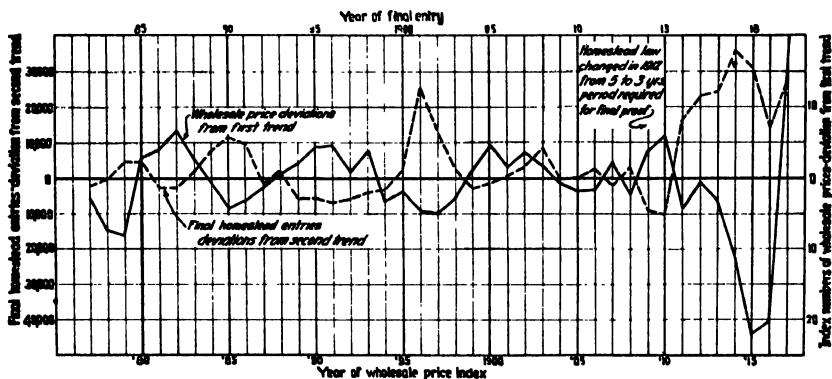y the inverse relationship between wholesale price of all commodities and final homestead entries lagged for the period between original and final entry. There was a tendency for a much larger percentage of original entries to become fi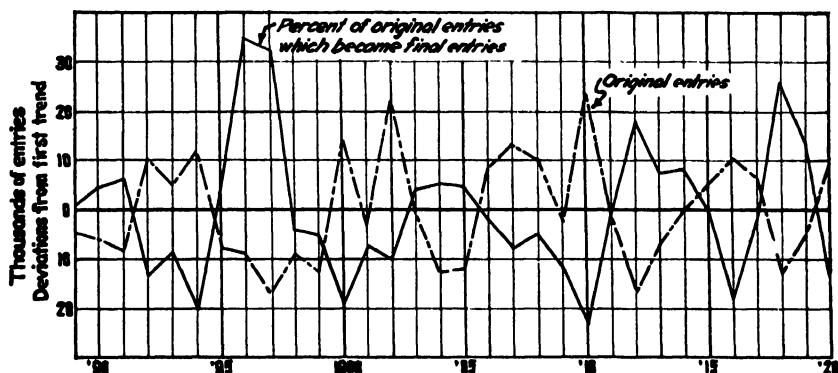nal when the total number of original entries was small. In other words, there was an inverse relationship between numbers of original homestead entries and the per cent of those which became final entries. This is shown in figure 13. Economic conditions at the time of entry and the total numbers making entry are not the only causes which influenced the percentage of the number of original entries which became final.
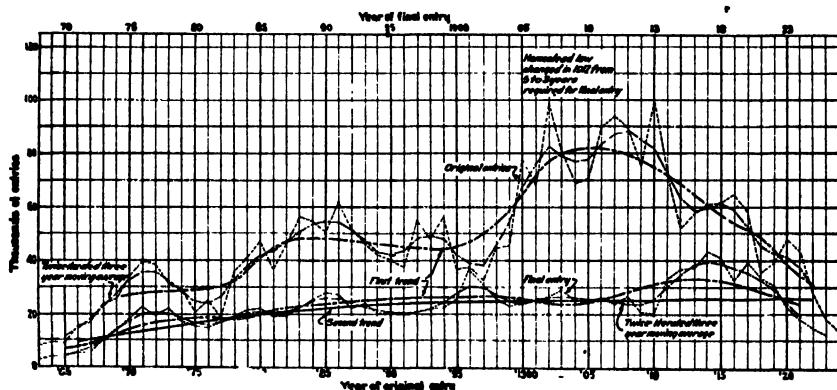
Fig. 10. Cyclical analysis of original and final homestead entries, 1863–1924.

Although obscured somewhat by the relationships already mentioned, there is an indication that economic conditions a year after entry have an important effect upon those remaining on their homesteads. It would be reasonable to suppose that this would be true with the whole-sale prices lagged two and one-half years or one-half of the period between original and final entry, for it would seem that business conditions during the period of settlement might affect the numbers who stayed on the farms. The results of the analysis, however, indicate that probably a large number of those giving up did so during the early part of their period of proof and their tendency to leave their homesteads was retarded by poor business conditions which probably meant lack of opportunity for employment in other fields than agri-

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[13] Farm economics. Dept. of Agr. Econ. and Farm Man. New York State College of Agriculture, Cornell University, Ithaca, N. Y. 45:698. June, 1927.

culture. There is some tendency toward the same relationship without the lag. In other words, high price conditions at time of original entry indicates a small percentage making final proof. The complex nature of the problem is brought about by the fact that the composite economic situation prevailing through the five- and three-year period of proof affected not only the numbers taking up land, but also the number leaving the claims during the period of proof.
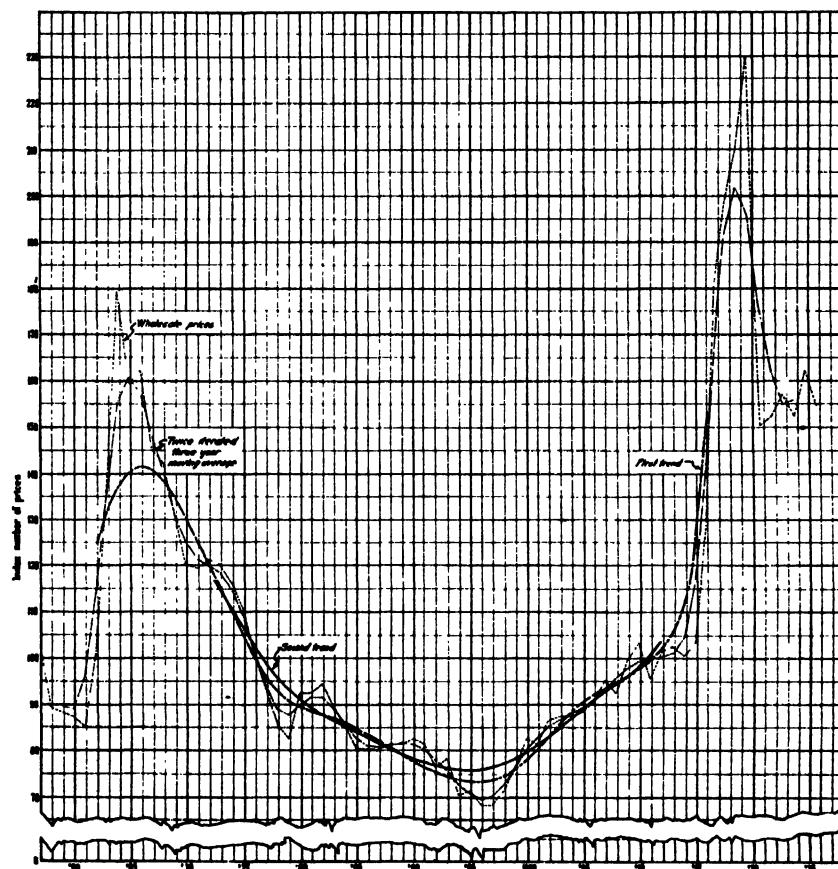
Fig. 11. Cyclical analysis of wholesale prices of all commodities, 1857–1927.

Factors Affecting the Economic Supply of Land.—Statistical analysis of economic supply of land have not been undertaken in the present study. Advertisements for farms, data on the character of reclamation projects and information concerning farms being purchased, furnish statistical measures of supply. There are at present about 22,000,000 acres in drainage enterprises in the United States

provided with reclamation which are not being utilized for agricultural production. About 7,000,000 acres though undeveloped are provided with irrigation facilities. These lands are available, but at a price which is sufficient to cover costs of the raw land and of the reclamation construction. This price may, therefore, be a limitation to the economic supply inasmuch as costs of production when land costs are included may be so great as to eliminate the possibility of any net income. In addition, there is a large area capable of producing crops without reclamation. This land, however, is for the most part of poor quality. Though these figures indicate large supplies of land, the effective economic supply is limited by cost of development in rela-

Fig. 12. There is an inverse relationship between wholesale prices of all commodities and final homestead entries when the latter are lagged for the period between original and final entry.

tion to expected income and available capital for development. Economic supply of raw land, unimproved, is, like the demand for undeveloped land, subject to different influences from the supply of improved land. Raw land is usually held by owners who are desirous of selling it at a price which will pay them for holding charges and development costs and usually an additional amount in the form of business profits. The growing scarcity of fertile soil in undeveloped lands tends to decrease this economic supply. Increasing costs of development, carrying charges, and overhead costs of agencies subdividing and selling undeveloped land tend further to decrease the effective economic supply. In irrigated areas, water supply is also an important influence upon the supply of irrigable land. Speculative irrigation enterprises spring up during times of business prosperity but are likely to be developed, if the price of land is low enough, during periods of poor industrial conditions. The recent war period

introduced exceptional influences which require caution in drawing conclusions. The supply of improved land is indirectly influenced by many of the factors which affect the supply of undeveloped land. The situation of the seller, however, is entirely different. The owner of the improved farm may be more or less willing to sell his farm according to different conditions which may prevail. High prices for agricultural produce may induce him to hold his land at a higher price per acre, thus limiting the effective economic supply. Opportunity for part-time employment in nearby urban centers may have the same effect as increased agricultural prices. However, attractive opportunities for industrial employment or desire on the part of the family for

Fig. 13. There is an inverse relationship between numbers of original entries and the per cent of those which became final entries.

modern conveniences and attractions, may increase the economic supply of land by reducing the price at which farmers, who govern a given land market, are willing to sell their farms. The peculiar characteristic of supply in this case is that the same conditions that would tend to cause an increased supply would tend to cause a reduced demand. Either one alone would reduce land prices. The effect of land price on rate of buying and selling and the reverse, the effect of rate of buying and selling upon land price, is pointed out by Gray and Lloyd who in describing the land boom of 1919 stated that "the increase in value tended to stimulate actively in buying and selling while this activity in turn reacted upon values."[14]

The relationship between factors influencing supply of and demand for land are indicated in the time series which have been analyzed. Frequently exceptions may be noticed to the inverse relationship men-

[14] Gray, L. C., and O. G. Lloyd. Farm land values in Iowa. U. S. Dept. Agr. Dept. Bul. 874:37. August 23, 1920.

tioned above between business conditions and movement to undeveloped land. A direct relationship between an index of business conditions and the rate of movement to undeveloped land would indicate that during prosperous times agriculture was still more prosperous because of attractive price conditions or from other causes. This occurred during the early part of the war period. During industrial depressions, agriculture might be even more depressed than industry, for although employment conditions in industry might be very unsatisfactory, the opportunity for agricultural returns might still be sufficiently poor to prevent a general movement to the land. This was the condition in the years immediately following the war. Income alone, therefore, from agricultural land is not the sole determinant of the most probable price which that land would obtain in a sale.

Land Prices and Agricultural Prices.—Wiecking,[15] in discussing the complex factors which enter into changes in land values, states that, "year to year fluctuations in earnings may not be reflected in values, at least, not immediately. Land yields its services year after year. One year's increase or decrease in income, therefore, may or may not affect value. Many considerations enter. How great the increase or decrease is, what its relationship to the trend over preceding years is, the extent to which it is considered more or less temporary or as an indication of the future trend, the general future outlook for earnings—these and other factors have.effect. It is probably the trend or average of income realized over a series of years which is the dominant influence on the earnings side. Even a reasonably stable trend in earnings, however, may be offset by other forces, of which a number are apparently still in operation." Black[16] in discussing the studies of income made by the National Bureau of Economic Research flays the method which was used in deflating inventory values to correspond to changes in prices. He states that "the preposterousness of this lies especially in the fact that land prices should not rise with the price level until it is clear that the new price level has come to stay." Chambers,[17] in discussing the effect of income on land value in the boom years of 1919 and 1920 states that land values "went up in these years in the main because land incomes went up, and because buyers of land did not discount the fact that these incomes were based upon

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[15] Wiecking, E. H. The farm real estate situation, 1926–1927. U. S. Dept. Agr. Cir. 15:7. Washington, D. C.

[16] Black, John D. Agriculture now. Journal of Farm Econ. 9(3):137–162. Bureau Agr. Econ., Washington, D. C. April, 1927.

[17] Chambers, Clyde R. Relation of land income to land value. U. S. D. A. Bul. 1224:38. Washington, D. C., 1924.

abnormal conditions." He further illustrates the tendency of land purchasers and sellers to inaccurately anticipate the future, by the following statement: "In the early part of this century, the farmers, who are the principal buyers and sellers of land, had fresh in their memories the long depression of the nineties. Furthermore, land incomes up to this period had not increased very much or very rapidly. Hence, very little was anticipated in the way of further increase in land income and the ratio of rent to value was therefore relatively high. But in the years following 1900 the average increase in land income was greater and greater, so that a constantly increasing percentage of the value was based upon anticipated increases in income, resulting in declining ratios of rent to value."

The fact that land prices are based upon inaccurate estimates of future returns introduces much irregularity in the relationship between prices of farm commodities and land prices. Much more important than changes in prices of agricultural commodities alone is the relation of that change to changes in costs. Forster[18] has shown that the remarkable increase in land prices in Kentucky in 1919 and 1920 was due not alone to the increase in tobacco prices but also to the fact that tobacco prices increased so much more rapidly than costs. He states that "while there was an increase of 269 per cent in the price of tobacco there was an increase of only 104 per cent in the cost of production." Gray and Lloyd[19] have emphasized this same cause of the 1919 land boom. In addition they mention the fact that "during this period farmers used many kinds of equipment bought at the earlier prices of the pre-war period, such as machinery, work horses, harness, etc." All of these writers have given much attention to the phenomenal increase in land prices during the period of inflation during and following the war, but little attention has been paid to general economic influences upon land prices under less extraordinary conditions. Since the war, the trend of profits from agriculture has been reversed. The trend of agricultural prices has been downward. The trend of land prices has also been downward. The relationship is not necessarily a simple illustration of cause and effect, however. In most of our studies, we have found different causes and effects, in regard to land price changes, in evidence during more normal times than occurred during the war inflation and deflation period. We have already mentioned the complex relationships existing between indus-

[18] Forster, G. W. Land prices and land speculation in the Bluegrass Region of Kentucky. Kentucky Agr. Exp. Sta. Bul. 240:64. Lexington, Ky.

[19] Gray, L. C., and O. G. Lloyd. Farm land values in Iowa. U. S. D. A. Bul. 874:3. August, 1920.

trial conditions, agricultural prices and land prices. The lag of agricultural costs behind agricultural prices during periods of increasing and decreasing prosperity adds to this complexity and diminishes our expectation of a close correlation between land prices and agricultural prices.
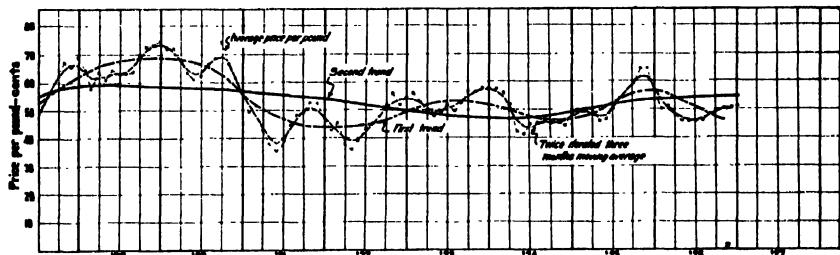
Fig. 14. Cyclical analysis of California butterfat prices, 1919–1926.
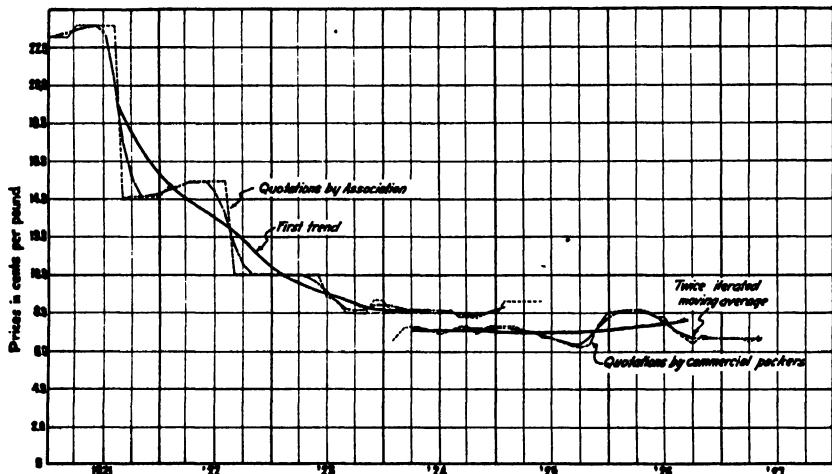
Fig. 15. California wholesale raisin prices.

There is some indication of a direct relationship between butterfat prices and price of land through the years 1923–1927, inclusive. A longer period would be necessary to verify this tendency. Through the years from 1919 to 1922, inclusive, however, there seems to be no relationship whatever between butterfat prices and land price. Figure 14 is a cyclical analysis of butterfat prices.[20] In the San Joaquin

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[20] Source of data:

    Voorhies, Edwin C. Economic aspects of the dairy industry. California Agr. Exp. Sta. Bul. 437:80. *Table 35.* 1927.

Valley where the price of raisins has been such a vital factor, there is little indication that fluctuations from year to year in land prices have been affected thereby. Figure 15 shows the wholesale price of raisins from 1921 to 1927.[21] It will be observed that control of prices upsets the cyclical tendency in the first trend. There are no cyclical relationships for short periods, at least, between raisin prices and land prices. There is, however, no question but that the secular trend of land prices in the San Joaquin Valley is greatly influenced by the raisin situation. This probably applies to lands other than vineyards. In the long run, crop prices undoubtedly are one of the important influences, but not the only one, governing land prices; but over short periods of time industrial or financial conditions seems to be much more important causes of variations than agricultural prices.

PRINCIPLE OF RELATIVE PRODUCTIVE VALUE

Quantitative determination of the effect of different land qualities combined in different proportions is basic to scientific appraisal. Factors influencing land value or price of land are so numerous and complex that if we are to resort to relationships as a guide to variability of price or value, we must simplify our work by segregating the important factors into groups and by analyzing the relationships within each of these groups before attempting to study relationships between land value and the indexes of the groups themselves.

The combining of a number of related variables into a single index seems to be the only hope of reducing land valuation to a scientific basis. The variables are so numerous that if they are considered separately and independently, rational interpretation becomes hopeless. To illustrate this important point the productivity of a piece of land may be taken as an example. Productivity is usually considered the most important factor in land valuation. In fact, the Federal Farm Loan Act dictates that productivity shall be the *basis* of land valuation. Productivity itself, however, is determined by a large number of other factors such as rainfall, temperature, soils and many other elements. The measurement of productivity in terms of the many elements affecting it would result in an index which would represent the net effect of these elements in the final analysis of value. It would be used as a single factor instead of many variables in correlations, thus simplifying a complex problem.

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[21] Raisin prices from which figure 15 was constructed were compiled by Dr. S. W. Shear, Division of Agricultural Economics, University of California.

Livingston[22] has done some work on the development of physiological indexes of productivity with special reference to climatic conditions and has given us a temperature index based upon the work of Lehenbauer.[23] The index developed by Livingston in his early work was merely intended to give the temperature efficiency for different parts of the United States. It is so general, however, that its value as a local index to be used in land valuation would be limited. Its chief value is to be found in general geographical studies. The work of Livingston and Lehenbauer, however, give us a suggestion of the possibility of combining the results of many variables into a single factor, thus simplifying a complicated study. Future work in this field should be coordinated with more recent researches of this character by these and other investigators.

A Corollary to the Principle of Proportionality.—The difficulty of such studies is increased by the fact that most of the factors affecting productivity have curvilinear relationships following the principle of proportionality. This principle has been stated in many different forms and is frequently called the principle of diminishing returns. Some economists believe that the use of the latter expression, "diminishing returns," applies only to the broader population problem; whereas when different combinations of elements of production are concerned the principle of proportionality is involved. Fetter in his *Economic Principles* states that, "a clear understanding of this most fundamental principle of proportionality. is essential to the solution of the complex problems of valuation. Things are not valued in isolation from each other. The great mass of complementary agents act and react upon each other. The valuation put upon one agent is due in part to the presence in certain proportions of other agents."[24]

Beckett and Robertson[25] have shown that the yield of alfalfa under the application of additional amounts of irrigation water follows the principle of proportionality. We have only to extend this principle to apply it in the evaluation of lands having different quantities of water available for irrigation. Just as each additional unit of fertilizer increases productivity up to a certain point after which returns

[22] Livingston, Burton Edward. Physiological temperature indices for the study of plant growth in relation to climatic conditions. Physiological Researches. 1913–15 1(1):399–420. Physiological Researches, Station H, Baltimore, Maryland.

[23] Lehenbauer, P. A. Growth related to temperature. Physiological Researches 1913–15 1(1)247–286. Station N, Baltimore, Maryland.

[24] Fetter, Frank A. Economic principles. 1:134. The Century Co., New York, 1926.

[25] Beckett, S. H., and R. D. Robertson. The economical irrigation of alfalfa in the Sacramento Valley. California Agr. Exp. Sta. Bul. 280:272–294. Berkeley. May, 1927.

per unit added decrease, land situated under different conditions of temperature vary in productivity according to the same principle.

Black has stated the principle of "diminishing physical outputs" as applied to several factors of production in combination, as follows: *"As increasing inputs of one or more elements of production are added to one or more fixed elements, a point is soon reached after which outputs per unit of the varying input elements decrease; and if more than two of either fixed or varying elements are involved, the points at which the decrease sets in, and the amount of all the outputs per unit of input, are affected by the inter-effects of the changes in the several varying elements, and also by the inter-effects between the several fixed and the several varying elements."*[26]

The above carefully worded principle, is probably a good starting point from which to develop a corollary for application to land value studies. Designed primarily for the analysis of production problems, it is clearly inadequate for the purpose of describing the variations in value of different tracts of land composed of qualities combined in different proportions.

In considering the productivity of one piece of land relative to that of another we are not adding factors of production in different proportions. We are simply comparing two different sets of such combinations, many of the elements in each being capable of little change. The underlying principle is the same but previous statements of it are inadequate for this purpose. A corollary to the principle of proportionality which is fundamental to land valuation and which may be extended in its application in other fields might be stated as follows: *In considering the relative productive value of two or more parcels of farm land it must be taken into consideration that the productive value of a given element or group of elements is dependent upon the proportion which that element or group of elements bears to the other essential elements contributing to or detracting from the productive value of the whole and that up to a certain point each unit of any essential element of productivity will be relatively more valuable as it is found in larger proportions but that superabundance of any element of productivity may result in a lower value per unit of the contributing element or may actually detract from the total productive value of the parcel of land in question.* In applying this principle it must be borne in mind that changes in supply and demand are held in abeyance and that time does not enter as a factor.

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[26] Black, John D.   Production economics.   p. 309.   Henry Holt & Co.   New York, 1927.

Furthermore, it is not intended as a substitution for the theory of rent although it is the belief of the writer that it has greater significance and usefulness in the practical problems of land appraisal. In referring to this principle in the following pages, it will be called the *principle of relative productive value.*

The relative productive value of one piece of land as compared to another will be greater, other variables being constant, for that land situated under conditions of higher mean temperature. This is true only up to a certain degree of temperature after which the land so situated that its mean temperature is still higher will tend to have a lower productive value. This principle applies in general to other land qualities, not only those affecting physical productivity but many of the economic qualities as well.

An Index of Productivity

The construction of a productivity index is complicated by the many influences affecting crop adaptation; by the fact that many crops do not utilize, for economic and physical reasons, the entire climatic efficiency available; by the fact that productivity is often created by methods of culture rather than by inherent qualities in the soil; and, by the fact that for economic reasons we do not find crops distributed over a sufficiently large range of conditions to afford an opportunity for adequate sampling. Even when the problem is reduced to its simplest form, that of the yield of a single crop produced throughout a fairly wide range of conditions, relationships between yield and the factors causing that yield are curvilinear. Not only are these relationships curvilinear, but the curves in some cases cross one another indicating that positive correlations between certain variables in one region become negative in another. Added to these difficulties are the large numbers of influences affecting yield which, in order to be studied separately, require the grouping of available data on yield and value into such small groups that the question arises as to the representativeness of available data with respect to the group which it is supposed to represent. In the face of these disturbing variables too much in the way of results can not be expected at first.

In a previous section the need of simplifying the analysis by grouping the indexes of productivity, community values, and other similar attributes have been discussed. It has also been seen that land qualities occurring in different proportions follow a corollary to the principle of proportionality which corollary, for convenience, has been

called the principle of relative productivity. Relationships following this principle are curvilinear and the variables are numerous. A large amount of data is necessary, therefore, for detailed analysis. An enumeration of only a few of the more important variables included in a study of yield as a measure of productivity indicates how large the number of groups becomes and how small the number of observations in each group must become. In such a study, farms must be sorted into groups according to soil characteristics, temperature, rainfall, several grades of quality of irrigation, a similar number of drainage conditions, varying degrees of alkali concentration, the presence of hardpan at various depths, the prevalence of weeds, pests, etc., with a view to studying yields in these respective groups. Average yields, therefore, due not only to soil conditions but to different degrees of personal efficiency, different amounts of capital invested in production and different seasonal conditions which vary from year to year are sure to have a high dispersion. Notwithstanding these wide variations, a definite tendency for different soil textures to have average yields which follow the corollary to the principle of diminishing returns has been established, and although a higher degree of accuracy may be expected as a result of future investigations, the present study gives a means of measuring productivity, under different general conditions, to a greater degree of certainty than has hitherto been possible in appraisal work.

In the Eleventh Federal Farm Loan District irrigation is of great importance in agriculture. Rainfall, except that it replenishes the irrigation supply, is therefore of less importance. Variations in rainfall may be expected to have little direct effect upon the productivity of farms having adequate water supply and depending almost entirely upon irrigation for moisture. Other factors, with the exception of temperature, which might be expected to affect productivity are capable of segregation. Even the effect of temperature may be studied by itself by grouping farms about a given temperature station. Yields of crops of different kinds may be studied in relation to the different factors affecting that productivity by eliminating as many variables as possible. When this is done and average yields determined, the principle of relative productivity has been found to hold. The present study has been greatly simplified by the elimination of all farms having accumulations of alkali, hardpan, less than first class irrigation or drainage conditions, weeds, pests or other unfavorable conditions. The elimination of these makes it possible to establish a standard for the measurement of the effect of such qualities in later studies. A

number of difficulties still exist however which cause some trouble in analysis. Elimination of these unfavorable influences diminishes the range of productivity which would otherwise exist. Whole soil series are eliminated. The number of cases is reduced to such an extent that there is considerable dispersion even of averages from regression lines. Even if the range in productivity were not reduced in this manner it would be limited by the fact that crops tend to be produced under conditions of more favorable productivity. Observations of productivity values cannot be obtained for soils far below the limits of profitable cultivation. This brings up the complication of adaptation which is so involved that it has been reserved for future study.

The Combination of Productivity Indexes for Different Crops.—It would be desirable to develop a productivity index which is a composite of the yield of a number of important crops. The combination of productivity indexes for different crops is complicated by crop adaptation. It is necessary to approach this question cautiously. A measure of the comparative advantage of two areas cannot be made upon the basis of yield alone. The economic tendency for a piece of land to produce up to certain limits that crop for which it is best adapted introduces another important factor, second only to yield, in determining the relative economic productivity of two or more different areas.

An explanation has been made of the necessity of limiting the scope of this phase of the analysis. Some of the reasons given for limiting the scope of the study also apply to a discussion of the difficulties to be met in constructing a productivity index which will serve as a measure under different conditions of crop adaptation. The yield of fruit lands is complicated by the influence of the age of orchard or vineyard. Furthermore, many soils unfit for alfalfa or other crops are utilized for highly productive orchards. Labor may be an important element in deriving this productivity. The same degree of productivity might be physically possible but economically impracticable in the case of a lower priced crop. With the production of such a crop as oranges, fertility may be created at a great expense with profitable results. In such cases, the value of the soil may be simply the opportunity it affords to the crop for standing room as intensity of culture introduces the needed elements of productivity. Because of the multitude of complicated factors, it has been considered safer at the present time not to launch an attempt to combine indexes of productivity based upon yields of different crops until a number of individual studies have been completed with regard to special types of agriculture.

The study of differentials in productivity has for this reason and for reasons discussed elsewhere been limited to a single product, alfalfa, which is the basic crop in the dairying industry. Dairying is being conducted on the farms included in the analysis, because it is probably in most cases the best of the alternative purposes for which the land might be utilized. It has not been assumed, however, that the dairying industry alone has determined the value of the land utilized for dairying, but has competed with other crops in the purchase of land. Ultimately, when similar studies of values of land utilized for other purposes have been made, it may be possible to clarify our knowledge of alternative use and crop adaptation with respect to their effect upon land values.

Limiting the scope of the study in this way has its disadvantages and therefore it may be necessary later in the light of more detailed and extensive studies, to change some of the conclusions. It is expected that future work will result in changes and refinement. A beginning must be made, however, and it is with this understanding that the results of this preliminary analysis are being presented.

An Index Based Upon Alfalfa Yields.—The indexes of productivity in the present analysis are merely the average yields of alfalfa growing on irrigated land with adequate water supply under different conditions of soil texture, different mean temperatures and different annual ranges of mean temperature. The fitting of curvilinear regression lines to these averages increases the reliability of the index. Irregularities caused by inadequate data in certain groups, but supported by adjoining groups of large numbers of cases are smoothed out with a most interesting result showing the application of the basic principle of diminishing returns to the relative productivity of different lands.

Alfalfa has been used because it is a crop that utilizes the entire growing season, because it is grown in all parts of the district, and because it is the principal feed for dairy cattle throughout this district. It therefore·lends itself to the study of dairy land price differentials used as an example of the application of the principles developed in this study. This index·should be valuable in the extension of the analysis to other types of land but complications become numerous as soon as other crops, especially fruits, are brought into consideration. Grain crops harvested in early summer may or may not utilize the entire temperature efficiency of a region. There may be economic justification for growing a crop which does not make full use of the available source of energy. Economic and

physical influences determine crop adaptation. It may be necessary to measure productivity with one or a few crops as with alfalfa, and introduce a new factor measuring adaptation when other types of agriculture are included in land price studies. Although grown generally throughout the district, alfalfa is not a universal crop. It is probable that it will be necessary to develop indexes by the use of crops which overlap in their soil adaptations. One of the difficulties in preparing the alfalfa index is that a given soil may in general be of low productivity, but under especially favorable conditions alfalfa may be found growing upon it, and yields obtained under these favorable conditions of growth may be represented in the correlations. For this reason, only soils have been included which are generally utilized for growing alfalfa.

Relation of Temperature to Productivity.—The study of effects of temperature upon plant growth in itself is not an economic problem. When we begin to consider the money value of different temperature conditions, however, we are well within the field of economics. There is a zone between the physiological and the economic phases of the subject which must be explored jointly by workers in both fields. If the writer has apparently gone beyond his depth in the physical aspects of the problem, it is only with a purpose of arriving at a point where it is possible to begin the economic analysis. Certain physical problems must be solved before the solution of the economic problems can be begun. Coordination of physical and economic studies will be necessary in future work in this field.

An attempt will not be made to review the literature on the effect of temperature upon plant growth. Lehenbauer[27] has given us some important findings in this respect which are very useful from the standpoint of economic analysis of factors affecting land price. Lehenbauer's curve showing the relationship between temperature and rate of growth has the typical characteristics of the principle of diminishing returns. Table 5 shows Lehenbauer's "mean average hourly growth rates (hundredths of a millimeter) for shoots of maize seedlings at temperature of from 12° to 43° C. The length of exposure in this case was 12 hours. The results of the work of Lehenbauer are used in the present study to give us the general shape of the temperature growth curve. That the factors determining the temperature of maximum growth rate and the magnitude of the maximum growth rate are variables is indicated by the results of Lehenbauer's experi-

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[27] Lehenbauer, P. A. Growth of maize seedlings in relation to temperature. Physiological Researches 1(1):247-286. Station N, Baltimore, Maryland, 1913.

ments, using exposure periods other than twelve hours, which resulted in optimum temperatures occurring at different degrees of temperature with corresponding different rates of growth at the maximum. "Indeed," states Lehenbauer, "it appears that the term 'optimum temperature' for growth in this case at least is quite without meaning unless the length of the period of exposure is definitely stated." Temperature efficiency for most practical purposes may be considered as a function of mean temperature and annual range. Under ordinary growth conditions, temperature is constantly fluctuating hour by hour, day by day, and year by year. The length of exposure becomes an erratic variable. Furthermore, different plants have different growth rates. The remarkable thing is that the shape of the Lehenbauer curve is retained at all owing to the caprices of natural conditions. Such is the case, however, when average alfalfa yields are plotted against mean temperature; the characteristic shape of the Lehenbauer curve is reproduced and it requires only a change in the vertical and horizontal scales of the diagram to practically duplicate it.

Effects of Daily and Annual Variations in Temperatures.—This similarity in the curves showing growth-temperature relationships is quite striking when it is taken into consideration that the mean annual temperature has within it all of the daily and annual variations which have different characteristics from region to region. The analyses show that daily range of temperature has little effect upon the alfalfa yield. This is possibly due to the observation noted by Lehenbauer that growth rates decline after a certain length of exposure and the effectiveness of the temperature of the day may be almost as great through a portion of the twenty-four hours as if it were continuous. Annual range, on the other hand, has a marked effect upon the yield of alfalfa. This would naturally be expected. A region having great range of temperature throughout the year is subject to shorter growing seasons and cooler winters. Coefficients of correlation of + .879 have been found between annual mean temperature and frost free period; of + .006 between annual mean temperature and daily range, and of − .539 between annual range in monthly mean temperature and frost free period. It seems that daily range in temperature has very little relationship with length of growing season. The correlations, among the three variables, mean temperature, annual range in mean temperature, and frost free period, indicate that it is not a serious omission to leave length of growing season out of the correlations. The coefficient of multiple correlation for these three variables was + .887.

Harmonic Characteristics of the Annual and Daily March in Temperature.—Daily and annual temperature variations follow very closely a modified form of the cosine curve. Figure 16 shows the mean monthly maximum and the mean monthly minimum temperatures of Phoenix, Arizona, with the curve fitted to the maximum series.

$$Y = 83.5 + 19.2 \cos. 30M$$

In this equation, Y is the mean maximum temperature, M is the number of the month of the year expressed in degrees numbering from July. One month is one-twelfth of 360° or 30°. July is 0° or 360°.
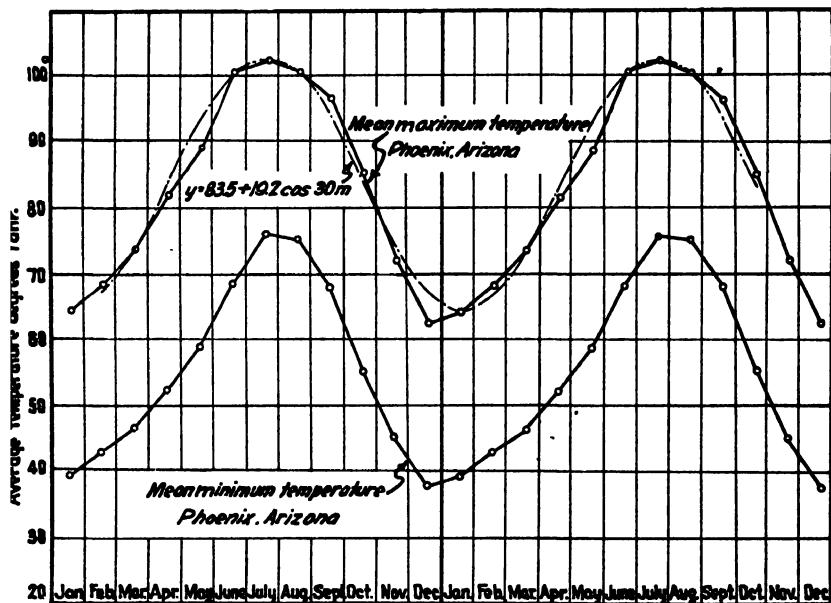
Fig. 16.   Tendency of temperature variations to follow the cosine curve.

Marvin[28] has found that for a section of the Northeastern United States, the annual curve of temperature may be represented by a single cosine curve. West[29] has given a formula

$$T = (\frac{Ma}{2} + Va \cos. t) + \frac{My}{2} \cos. t$$

"The constants are the mean annual temperature (Ma), the range of the annual march (Va), and the range of the daily march (My)."

[28] Marvin, C. F.   Are irregularities in the annual range of temperature persistent?   Monthly Weather Review 47(8):544. 1919.

[29] West, Frank L.   A simple equation of general application for the normal temperature in terms of the time of day and the day of the year.   Monthly Weather Review 48(7):394–396. 1920.

For a given station, however, the mean temperature for any day in the year can very closely be approximated by the simple cosine relationship

$$T = M + \frac{R}{2} \cos. \theta$$

where M equals mean daily temperature, R equals annual range in temperature, and θ equals time in degrees counted from the maximum.

Application of Results of Maize Growth Experiments and Harmonic Characteristics of Temperature Variations in Constructing a Productivity Index.—Because of the large number of possible combinations of mean temperature and range in temperature, the small number of cases of yield data in some of the groups makes it difficult to construct these curvilinear correlations without the aid of the results of Lehenbauer's studies and calculations based on the harmonic characteristics of the annual march of mean temperature. The annual temperature efficiency of a given climatological station may be considered as the sum of the temperature efficiencies for the different periods of the year. This is not exactly true because a given temperature is probably more effective for plant growth at one time in the year than at another. We can proceed with the assumption, however, that there will be a fairly high correlation between temperature growth efficiencies for a whole year integrated from the temperature growth efficiencies for the different parts of the year, and yield.

On the basis of the simple cosine formula given above, mean daily temperatures have been computed for 24 intervals of time throughout the year for annual ranges of temperature from 0° to 45° F and for annual mean temperatures from 25° F to 100° F. For each of these computed values of temperature, the corresponding growth rate found by Lehenbauer has been taken from table 5. Averaging these 24 bi-monthly values for a given mean temperature, it has been possible to obtain indexes of temperature efficiency for the year based upon mean hourly growth rates of maize seedlings for ranges of temperatures from 0° to 45° F. Figure 17 and table 6 are based upon the Lehenbauer experiments and upon the corrections for range in temperature described above. The curve having a range equal to zero is based upon the original Lehenbauer twelve-hour exposure experiment. In the cooler regions, for a number of months, the temperature efficiency is 0. Temperature efficiency is greater during the winter months for those stations having the longer growing seasons. Average temperature efficiency for the year is therefore greater for those stations having the longer growing season.

TABLE 5

MEAN AVERAGE HOURLY GROWTH RATES—HUNDREDTHS OF A MILLIMETER—FOR
SHOOTS OF MAIZE SEEDLINGS FOR A TWELVE-HOUR PERIOD OF EXPOSURE

(1) Temperature, deg. C.	(2) Growth rate*	(3) Temperature, deg. F.	(4) Growth rate†	(Col. 3, cont.) Temperature, deg. F.	(Col. 4, cont.) Growth rate†
12	9	40	0.0	76	72.896
13	10	41	0.3	77	76.601
14	16	42	0.6	78	80.446
15	20	43	0.9	79	84.439
18	28	44	1.3	80	88.561
20	45	45	1.6	81	92.753
21	53	46	2.2	82	96.924
22	59	47	2.7	83	100.927
23	64	48	3.4	84	104.554
24	69	49	4.2	85	107.241
25	75	50	5.1	86	108.630
26	82	51	6.0	87	109.455
27	90	52	7 1	88	109.804
28	98	53	8.2	89	109.338
29	105	54	9.586	90	107.916
30	108	55	10.916	91	105.564
31	109	56	12.546	92	102.361
32	111	57	14.376	93	98.380
33	101	58	16.501	94	93.752
34	97	59	18.879	95	88.637
35	86	60	21.430	96	83.084
36	74	61	24.127	97	77.183
37	70	62	26.947	98	71.010
38	58	63	29.872	99	64.618
39	46	64	32.887	100	58.059
40	31	65	35.979	101	51.400
41	20	66	39.136	102	44.706
42	11	67	42.346	103	38.063
43	6	68	45.606	104	31.573
		69	48.915	105	25.352
		70	52.251	106	19.535
		71	55.604	107	14.316
		72	58.969	108	10.009
		73	62.349	109	6.890
		74	65.853		
		75	69.321		

* Marvin, C. F. Are irregularities in the annual range of temperature persistent? Monthly Weather Review. **47** (8): 276. 1919.

† Growth rates are calculated from adjusted second differences taken from a smoothed curve fitted to the original data of column (2). Values in column (4) are given to three decimal places to make possible the reproduction of the smoothed second difference curves from which they were derived without cumulative error. In much work of this kind, differences in growth or rates of change are more important than absolute rates. Rates of change (first differences) and acceleration (second differences) may be computed from column (4). The number of decimal places should not be taken as an indication of the accuracy of any individual observation although an estimate from column (4) should be a closer approximation to the probable growth rate than any individual observation taken from column 2.
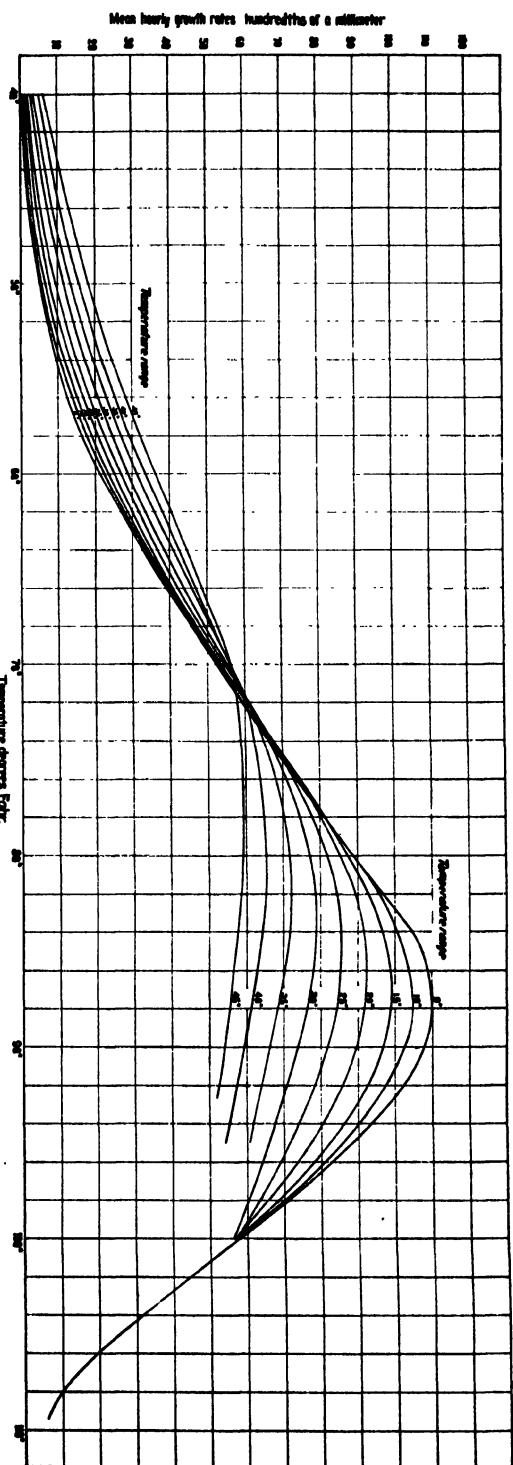
Fig. 17. Lehenbauer has derived mean hourly growth rates of maize seedlings under constant temperature conditions. The results of his experiments under conditions of 12-hour exposures are shown above in the curve for temperature range of 0°. This curve is a smooth curve fitted to his data. The data of Lehenbauer have been applied here to measure the growth efficiency of a given mean annual temperature associated with a given range in mean temperature throughout the year. The range as actually used is the difference between the highest monthly mean temperature and the lowest monthly mean temperature. The ordinates of the other curves of the illustration were obtained as follows: The ranges in the mean annual temperature,

M, from 0° to 45° F were used to establish the constant R in the cosine curve $T = M + \dfrac{R}{2} \cos \Theta$, which expresses the annual march in daily mean temperature, T. Time is measured by Θ. By means of this formula, mean daily temperatures were computed for 24 equal intervals of time during the year. Maize growth rates corresponding to these temperatures were averaged to obtain the ordinates of curves for different ranges in temperature. With the exception of the original Lehenbauer curve, these should not be considered as results of observations but as purely abstract indexes of the growth efficiency corresponding to mean temperatures for given ranges in monthly mean temperature. The complex variations in the growth of plants under the cumulative effects of changing temperatures are, of course, not exactly indicated by these indexes. Correlations, however, indicate fair approximation to growth conditions and therefore justify their use.

TABLE 6

TEMPERATURE INDEXES FOR USE IN CALIBRATING THE RELATIVE PLANT GROWTH EFFICIENCY FOR WEATHER BUREAU STATIONS IN THE ELEVENTH FEDERAL FARM LOAN DISTRICT

Range in temp., deg. F.	10°	15°	20°	25°	30°	35°	40°	45°
Mean temp., deg. F.								
25						.1	.2	.5
30				.1	.3	.6	.9	1.5
35		.1	.3	.6	1.1	1.7	2.4	3.3
40	.5	.9	1.4	2.0	2.8	3.8	5.1	6.2
45	2.1	2.7	3.6	4.6	6.1	7.8	9.7	11.8
50	5.6	6.5	7.8	9.5	11.5	13.7	16.0	18.4
55	12.1	13.4	15.1	17.0	19.1	21.4	23.9	26.5
60	22.4	23.6	25.2	27.1	28.9	31.1	33.6	36.3
65	36.6	37.1	38.0	34.3	41.1	43.3	44.8	46.9
70	52.4	52.7	53.5	54.5	55.2	56.2	56.1	55.2
75	69.9	70.8	70.9	70.4	69.0	66.5	63.2	59.0
80	88.7	87.4	85.2	81.8	77.1	71.6	65.6	59.2
85	102.3	97.8	92.0	85.4	78.5	71.1	63.8	56.7
87.5	104.9	99.0						
90	102.8	96.9	89.6	81.8	73.9	66.2	59.7	54.3
95	85.8	82.2	77.2	71.5	65.7	60.7		
100	57.4	57.0	56.7	56 8	56.2			
105								

From this point, the fact that the curve is based upon growth rates of maize seedlings ceases to be significant. The objective is to obtain a chart which will express the relationship between mean temperature and probable alfalfa yield for different ranges in mean temperature. Such a chart will obviously have the same characteristics as figure 17, but since the growing conditions of alfalfa are different and the units and basis of measurement are different, the vertical and horizontal scales will be different. If it were not for the variability introduced by range in temperature we should be able to construct the desired chart from the alfalfa yield data alone. It is a measurement of the effect of range in temperature which compels us to use the following method of calculation. By changing the vertical and horizontal scales, the curve showing the relationship between the average yield of alfalfa on sandy loam under different conditions of mean temperature can be made to conform to the 30° range line of the maize-growth curve in figure 17, 30° being approximately the average range in temperature for the cases of alfalfa yield included in the correlation. This was accomplished by the following procedure. The horizontal scale of the 30° range line of the maize-growth curve was changed to bring

the maximum of that curve into the same vertical line, as the maximum of the curve of sandy loam alfalfa average, 40° mean temperature being considered identical in both curves. This was approximated at first by observation of the points of maxima. The adjustment was refined finally by adopting that horizontal scale which would give the highest correlation between alfalfa yield averages and maize-growth corresponding to mean temperature on the adjusted horizontal scale. By considering both scales coincident at 40° mean temperature, the temperature of maximum efficiency for corn of 84° was reduced to 68° for alfalfa. By such adjustment of the horizontal scale it was possible to obtain a correlation of + .67. It must be remembered, however, that temperature range is still a cause of dispersion. By means of the regression line of this correlation a new vertical scale was derived. This regression line is an expression of the relation between the values on the vertical scale of the maize-growth curve and the values on the vertical scale of the alfalfa yield curve. The resulting curve shows mean temperature-alfalfa yield relationships, average temperature range being 30°. By means of the new vertical and horizontal scale adjustments, the temperature-range corrections of figure 17 were plotted, as deviations from the 30° range line. In the original form this gave us a chart quite similar to figure 17, but for use in computations it was changed to the form shown in figure 19 in which the sandy loam alfalfa yield curve for a mean temperature range of 30° was reduced to a horizontal position. The other curves of this figure give deviations in the yield of alfalfa for different ranges in mean temperature corresponding to different mean temperatures.

Having obtained the basic sandy loam yield curve, the next step was to compute the respective deviations for the several soil textures from the sandy loam estimates. This was done by making an estimate of yield for each temperature station on the basis of the sandy loam alfalfa yield curve. These estimated values were subtracted from the observed yields for the different soil textures corrected for range in mean temperature by values read from figure 19. These deviations for each soil texture were sorted into groups according to class intervals of mean temperature. Average deviations were plotted for each mean temperature class and a smooth curve was drawn through these averages. These curves gave a basis for estimating corrections in yield for soil texture to be applied to estimates based on the standard sandy loam mean temperature yield curve. These estimated deviations measured in the proper direction above and below the sandy loam curve gave a set of curves similar to those shown in figure 18. Even
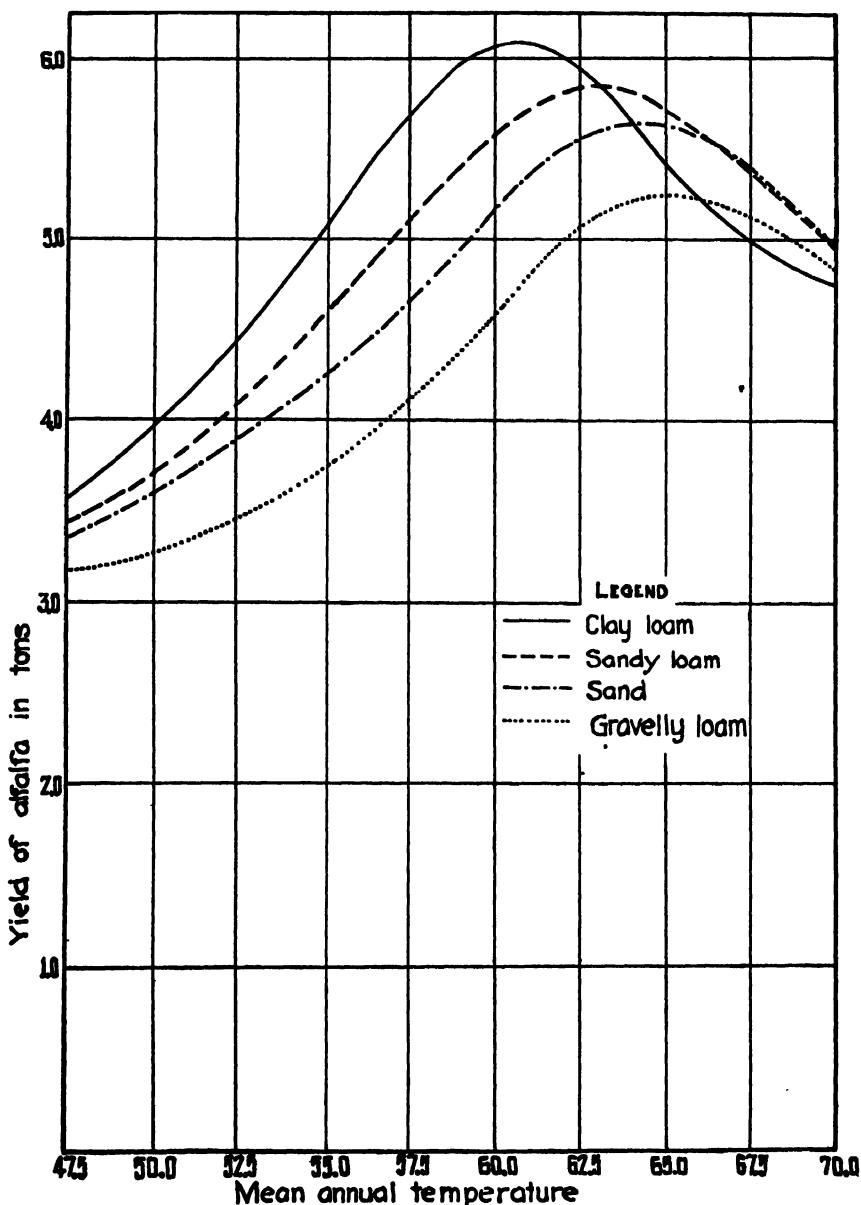
Fig. 18. Average yield of alfalfa for mean annual temperatures from 47.5° to 70° F for a range of 30° between the lowest monthly mean and the highest monthly mean temperatures.

the basic sandy loam curve itself was refined when studied in connection with deviations due to range.   Having individual characteristic curves for a number of the important textures, it only remained to take out the irregularities which were obviously erratic.   The curves were smoothed with the objective of obtaining continunity not only with respect to a single texture but from one texture to another.   The resulting curves for four important textures are shown in figure 18.[30]
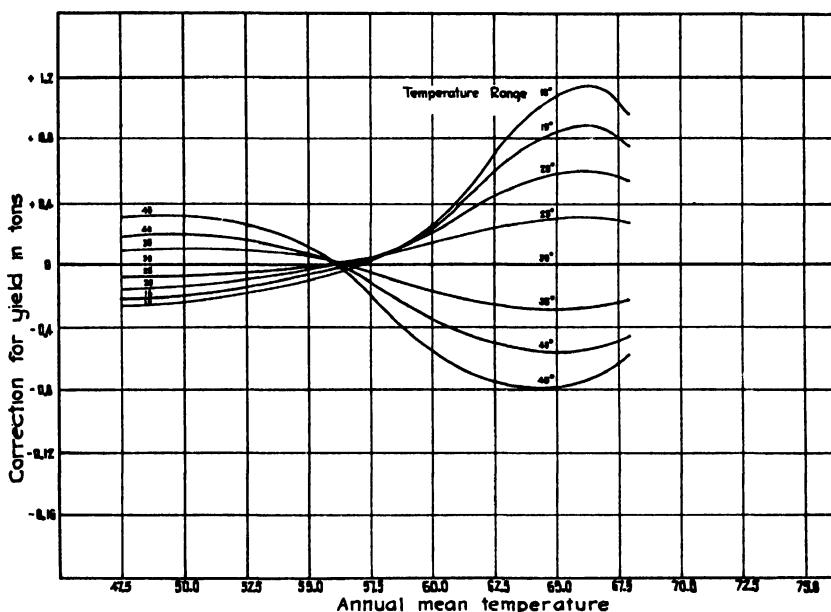
Fig. 19.   Corrections for annual range in temperature degrees Fahrenheit to be applied to alfalfa yields estimated on the basis of relationships of mean temperature to yield shown in figure 18.

It must be understood that these observations take into consideration only the average resultant effects of soil textures which in natural conditions are subject to many interacting effects of temperature, moisture and of aeration.   The subdivision of these into their components is truly the work of the soil physicist, while the difficult problem of ever-changing chemical conditions must be the subject of

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[30] The test as to the reliability of the method of analysis was made by correlating estimated yields of alfalfa with observed yields.   Estimated yields on sandy loam for the mean temperature prevailing at a given climatological station but for a mean temperature range of 30° F were paired with average observed yields at the same station corrected for range in mean temperature and for soil texture.   This correction was made to bring the estimated yields and the observed yields to the comparable basis of 30° F range in mean temperature and sandy loam soil before correlations were made.   The success of the method was established by a correlation coefficient of + .886.

detailed research by the agricultural chemist and plant physiologist. Estimated yields of alfalfa based upon figures 17, 18, and 19 are the productivity indexes used later in the land price analyses.

The Importance of Rainfall.—Inasmuch as this study does not include farms depending entirely upon rainfall for their productivity, later studies should include such analyses at which time much light may be thrown upon the effect of rainfall upon unirrigated portions of irrigated farms.

Price of Land in Relation to Size of Farm and Value of Buildings

Small farms sell for higher prices per acre than large farms. Cost of subdivision and sale, characteristics of demand for and supply of land, productivity, opportunity for employment off the farm, residence value, psychic values, including attractiveness of urban centers, and type of agriculture are elements in the size price relationship. Other elements are cost of farm development, scarcity of land of suitable location, building values, and total capital required for purchase. Some of these are causes of higher prices per acre. Others are effects. Some are both cause and effect indirectly reacting one upon the other coming into equilibrium with a resultant price which is higher in the case of smaller farms. Cost of subdivision and sale is greater for the small farm. Surveys, the making of records, and salesmen's fees are all practically as great for the small farm as for the large one, making the cost per acre greater. Can it be said that higher cost of subdivision and other selling costs are a cause of higher price or the result of a higher price? An answer to this question would involve a discussion of the theory of value as applied to land and improvements. This would require an analysis similar to other price and cost of production studies. Certainly higher costs of subdivision and sale are made possible by a demand at a price sufficient to cover costs. If it were not for these costs, however, the supply of small farms at a given price would be greater and the price would fall. High-valued lands tend to be cut up into smaller parcels. The soils in any large tract of land are likely to be poorer, on the average, than on a small farm. There is an economic as well as a physical reason for this fact. If small farms were carved at random out of large tracts of land, the average quality of the soil would probably be the same. A small farm, however, must have good soil or it will cease to be cultivated as a small farm. Five acres of poor land will have much greater influence upon the buyer of the 15-acre farm than upon the

160-acre farm. It is quite difficult to find a large tract of uniformly high quality land whereas land of very high degree of productivity may be found in small areas.

Although the price per acre is greater, the total amount paid for the small farm is less than for the large farm. The demand, therefore, is influenced by the amount of money possessed by purchasers, in relation to the total amount asked for the land. The total amount of capital required therefore being smaller for small farms is a factor determining a higher price per acre.

By the application of more labor per acre, though less might be received per unit for the labor, land will be made to yield more and a certain amount of the labor income is likely to be capitalized into the price of the land. So long as a living can be produced, a possibility of success will induce the seller of the land to demand "what the traffic will bear." This is true of farms large and small but in the case of small farms, the amount of such labor income so capitalized may be much more per acre for such labor is spread over a smaller number of acres. The capitalization of labor income into land price, then, is another element making small farms sell for a greater price per acre.

Small farms frequently are not the only source of income. If there is an outside source of income, a higher price can be asked for the land not only because of its residence value but because there is a joint source of income from the farm and from the outside source. The price per acre is thus determined by the advantage gained by these combined sources of income.

Small farms are frequently near urban centers, and the value of the small farm is increased not only because of its residence value and speculative value, resulting from anticipation of future urban expansion, but because of many economic advantages such as demand and marketing facilities for high-priced crops. High-priced crops make possible a living on small acreages. Intensity of culture is increased because of the higher value, and higher values result from intensive culture.

Figure 20 shows the relation of size of farm to appraised value of land and of buildings and also shows the relation between size of farm and the combined value of land and buildings. The average values from which this figure has been constructed are given in table 7. Figure 21 gives the same relationships between size of farm and resale prices of land and buildings in the San Joaquin Valley, California.
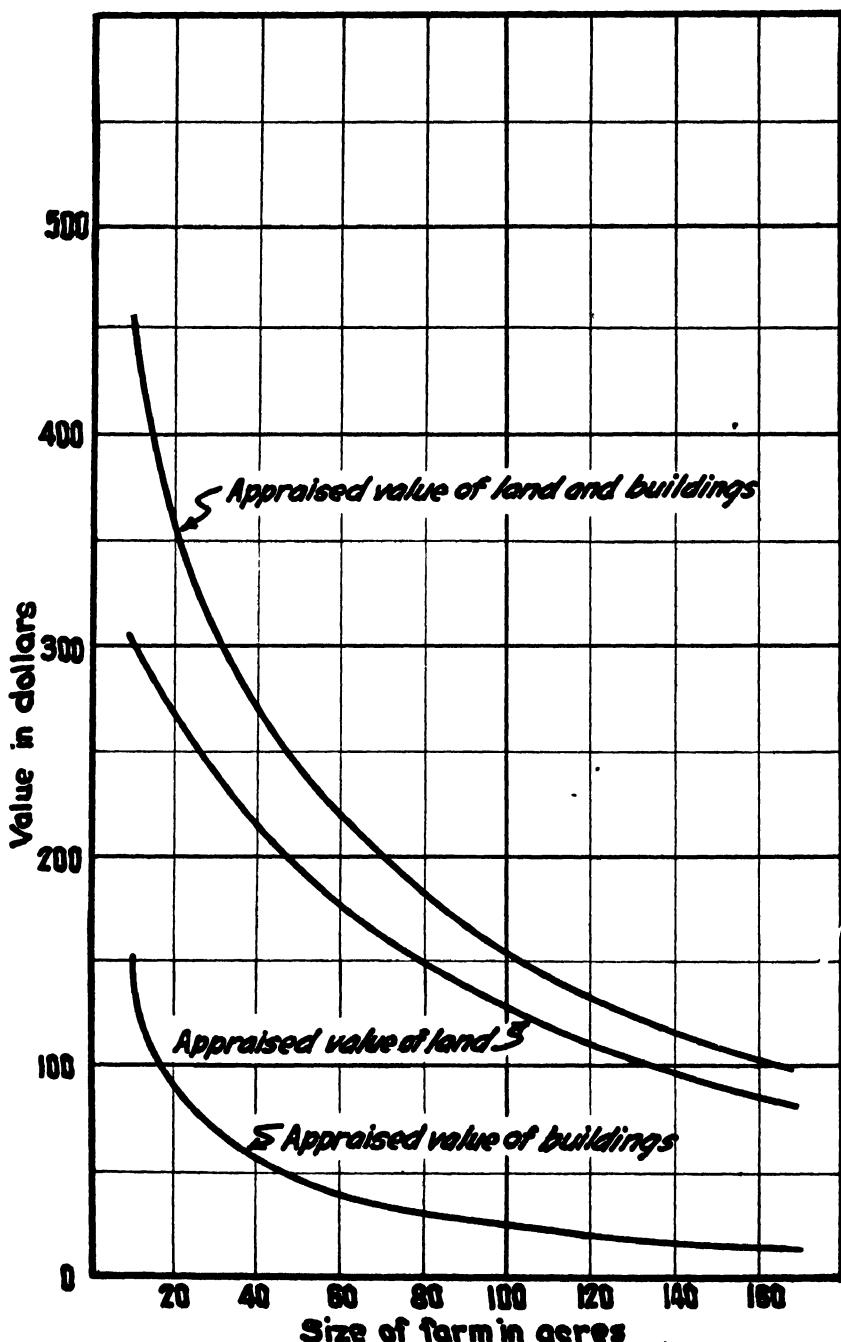
Fig. 20. Relation of size of farm to appraised value of land and buildings.

The average resale prices are computed from all available resale prices for farms on record in the bank for the sizes given. These average resale prices are given in table 8. Prices from which figure 21 was constructed were not deflated. The curve as shown, however, follows very closely the curve which would have resulted had deflated prices been used.
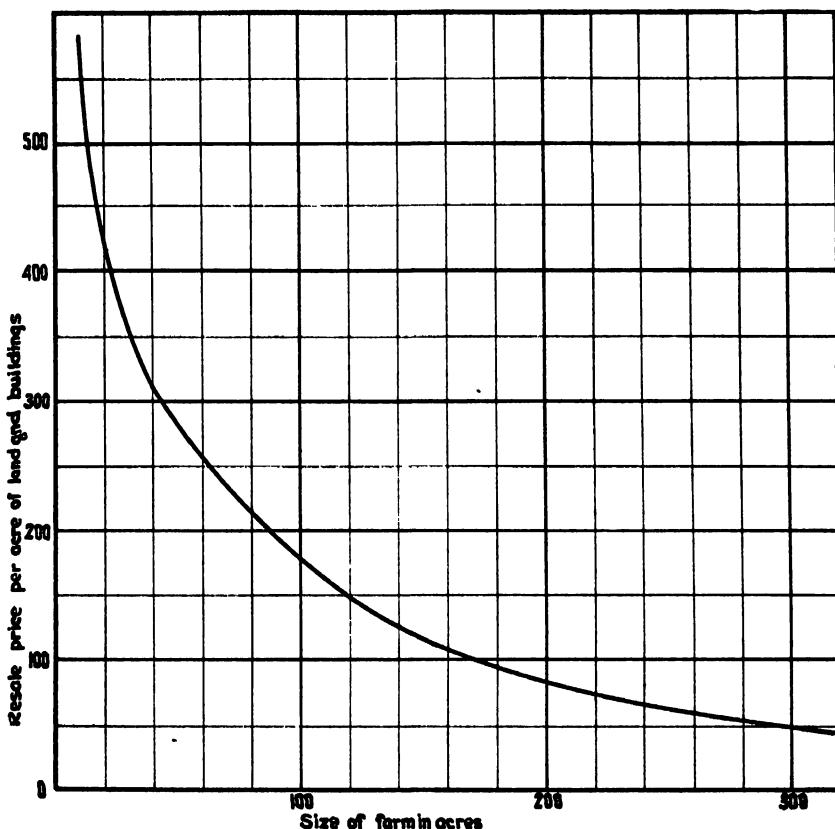
Fig. 21. Relation of size of farm to resale price per acre of land and buildings.

Figure 22 shows the changes which have taken place in the relationship of farm size to building value over the period of years from 1918 to 1927. Figure 23 shows the trend in appraised value of land for 10-, 20-, 40- and 80-acre farms from 1918 to 1927. Building values per acre have increased due probably to added volume of building. Land value trends for the various size of groups shows a consistent maintenance of a fairly constant general level.

TABLE 7

AVERAGE APPRAISED VALUES PER ACRE FOR LAND AND BUILDINGS ON FARMS OF
DIFFERENT SIZES COVERED BY FEDERAL FARM LOANS IN THE
ELEVENTH FEDERAL FARM LOAN DISTRICT, 1918–1926

Size*	Frequency	Average building value per acre	Average land value per acre
Acres		*Dollars*	*Dollars*
10	549	152	302
20	1601	90	245
40	1276	56	212
80	477	31	161
120	161	20	109
160	328	16	90

* A few farms slightly larger and smaller than the indicated size were included in the averages in
each group. Since most of the farms in each group are exactly the size indicated the average is highly
representative of the size group in each case.

TABLE 8

AVERAGE RESALE PRICES PER ACRE FOR FARMS OF DIFFERENT SIZES COVERED BY
FEDERAL FARM LOANS IN THE SAN JOAQUIN VALLEY, CALIFORNIA

Size*	Frequency	Average
Acres		*Dollars*
10	49	582
20	190	436
40	128	311
80	25	216
160	16	108
280	5	61
300	9	34

* Farms slightly larger and smaller than the indicated size are included in the averages of each size
group.

Building Value per Acre in Relation to Size of Farm.—The mathe-
matical relationship between size of farm and value of building
becomes a most confusing element in the interpretation of the purely
economic relationships between these same elements. The mathe-
matical relation between building value per acre and size of farm can
be postulated by holding land value per acre constant and total build-
ing value constant. If we denote total building value as K, building
value per acre as B, the size of farm in acres as S, the per acre value
of buildings may be expressed as follows:

$$B = \frac{K}{S} = K\left(\frac{1}{S}\right)$$

Building value per acre, economic and other physical forces being held constant, varies with the reciprocal of the size of farm. The reciprocal curve has the characteristic form of the rectangular hyperbola. Table 9 gives values of $\frac{1}{S}$ for sizes of farms, ranging from 10 to 160 acres. In the same table are given building values per acre corresponding to total building values ranging from $1000 to $3000. For each total building value the size intervals range from 10 to 160 acres. This table is designed to show the purely mathematical relationships between size and building value per acre.
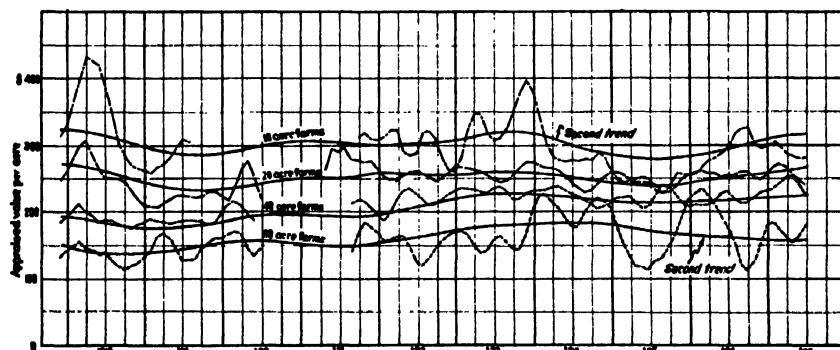
Fig. 22. Appraised value of buildings per acre on 10-, 20-, 40-, and 80-acre farms covered by loans in the Eleventh Federal Farm Loan District.

TABLE 9

RECIPROCALS OF FARM ACREAGE AND VALUES OF BUILDINGS PER ACRE, TOTAL
BUILDING VALUES AND FARM ACREAGES VARYING

S*	$\frac{1}{S}$	B when K=1000	B when K=1500	B when K=2000	B when K=2500	B when K=3000
10	.10000	100.00	150.00	200.00	250.00	300.00
20	.05000	50.00	75.00	100.00	125.00	150.00
40	.02500	25.00	37.50	50.00	62.50	75.00
60	.01667	16.67	25.00	33.33	41.67	50.00
80	.01250	12.50	18.75	25.00	31.25	37.50
120	.00833	8.33	12.49	16.67	20.82	25.00
160	.00625	6.25	9.37	12.50	15.62	18.67

* S=Size of farm.
B=Building value per acre.
K=Total building value.
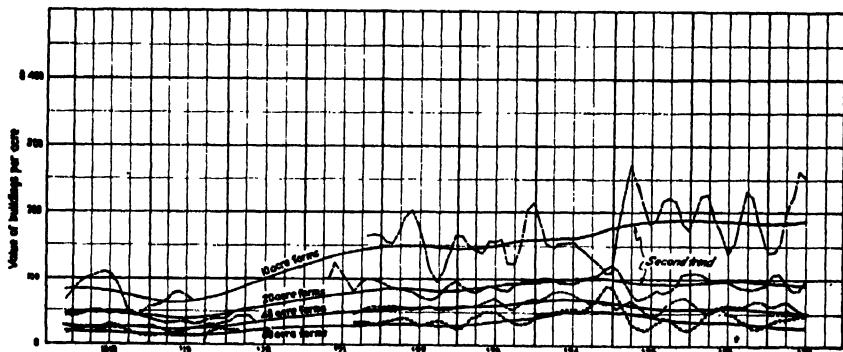
Fig. 23.   Appraised value of land exclusive of buildings in 10-, 20-, 40-, and 80-acre farms covered by loans in the Eleventh Federal Farm Loan District.
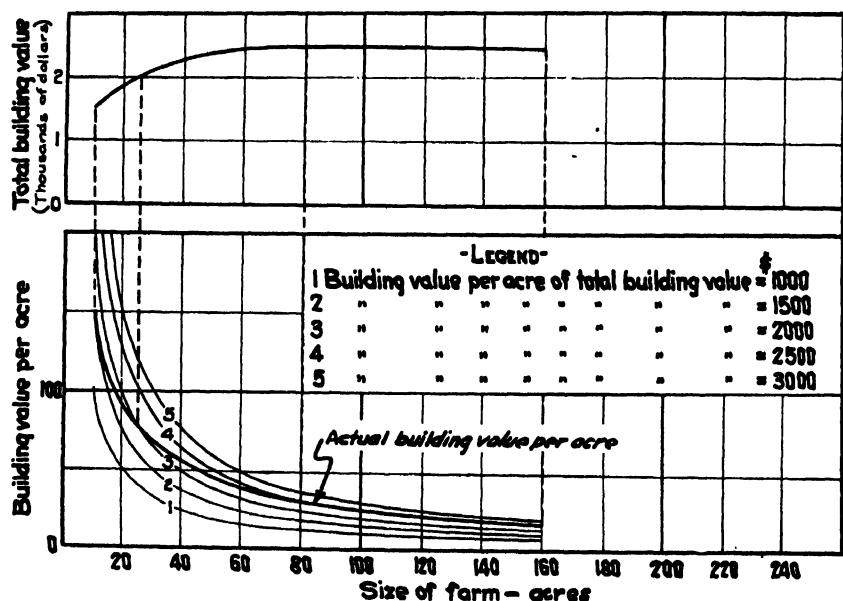
Fig. 24.   Mathematical and economic causes for characteristic shape of the curve of relationship between size of farm and building value per acre.

In figure 24, the total value of buildings for different sized farms is introduced as a variable. This variability is due to economic causes. Small farms tend to have smaller total building values. This may be due to the fact that less building equipment is necessary to carry on the farm operations on the small farm; it may be due to the fact that the earning power on small farms is not sufficient to provide such expensive buildings as on the larger farms; or it may be a combination of both of these. In the illustration referred to, the mathematical and economic aspects are clearly distinct. The light lines in the lower part of the drawing give the mathematical relationships between building value per acre and size of farm for total building values varying from $1000 to $3000. The curve in the upper part of the chart shows the average total appraised value of buildings on farms of different sizes.[31] Curvilinearity in this graph is due to economic causes. For the size of farm where this curve crosses the line of $2000 total building value, the heavy line in the lower portion of the chart crosses the mathematical curve of relationship between building value per acre and size of farm, total building value per acre being held constant at $2000. This heavy line is the curve of relationship between building value per acre and size of farm where both mathematical and economic influences enter. It is based upon average building values given in table 7. It is identical to the building value curve of figure 20.

Building Value per Acre in Relation to Value of Land and Buildings per Acre.—In the ordinary application of the principle of proportionality, we are familiar with the fact that as additional amounts are expended for buildings on a given farm, the return per unit of farm building value added increases up to a certain point after which the returns per additional unit decrease. The addition of buildings to a single farm may increase the per acre value of that farm in a greater proportion than the added building value per acre. After a time, successive additions will result in increased value of land and buildings per acre, but the increase in value of land and buildings will not be so great as the additional building value per acre. Finally a point is reached where over-development of buildings actually reduces the value per acre of land and buildings. A hypothetical case will illustrate this point. There are two unimproved 40-acre farms exactly alike in soil, topography, irrigation and drainage conditions, etc., lying on opposite sides of the highway so that they are the same distance

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[31] Averages of appraised values of all farms of indicated size covered by active loans in the Federal Land Bank of Berkeley, 1927.

from market, and situated in an excellent dairy section. They are held at the same price, namely, $250 an acre. Brown buys one of them and Smith the other, and both proceed to build and make their farms into ideal dairy farms. Brown builds an $8000 or $10,000 house and expends about $20,000 in barns and other buildings, so he has all the necessities and modern conveniences found on the best dairy farms. Yet his building is not "over-developed." Brown's farm now stands him $40,000.

Smith has plenty of money and likes fine buildings, so he builds a $120,000 mansion, and puts about $70,000 into barns, etc., making his farm stand him $200,000. Now Smith's building would probably be regarded as over-developed.

In the course of three or four years, Mr. Jones decides to buy a dairy farm and learns that these two farms are for sale. ' Brown is ready to quit dairying and offers his place for $42,000. Smith has died and his heirs do not care for farming of any kind so they offer their farm for $45,000 or even $43,000. Which, farm, if either, will Mr. Jones buy?

The net income from Smith's farm will be less, because of higher taxes, greater depreciation and other operation costs. The number of prospective purchasers for such an estate are few, and unless the location is such as to give the farm an especially attractive residence value, it is likely to be sold for the value of the old lumber in its buildings after the cost of salvaging the same has been deducted. This subject is discussed again on pages 519 and 524 in connection with figures 25 and 26. In the valuation of farm real estate, comparisons must be made of the relative values of different farms each having building values which may exist in widely different proportions to the total value.

Taking the farms as they are found with the buildings already constructed, the relative values of these farms vary with respect to each other according to the principle of relative productive value stated above. In other words, as applied to building values, if one considers farms which are similar in every respect except for building value, those farms which have the higher expenditure per acre for buildings have a more than proportionally higher value per acre for land and buildings combined, but this is true only up to a certain degree of development after which a piece of land may have less than a proportionally greater value per acre, while the farm greatly over-developed in buildings may actually have a value per acre less than that of a farm having a smaller expenditure per acre for buildings.

Figure 25 shows the relation of appraised value of buildings to appraised value of land and buildings on 20-acre farms covered by loans in the Federal Land Bank of Berkeley. When the size variable is held constant, the characteristic curve of the principle of diminishing returns is in evidence. Table 10 gives the average values upon which the curves in figure 25 are based. On farms having build-
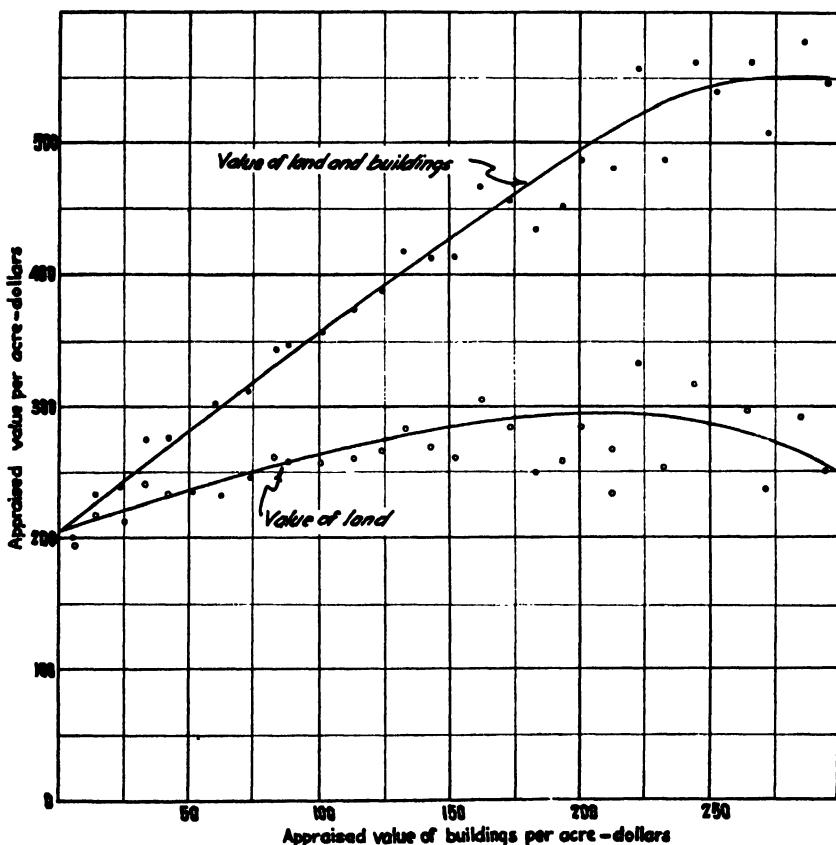

Fig. 25. Relation of appraised value of buildings to appraised value of land and buildings on 20-acre farms covered by Federal farm loans in the Eleventh Federal Farm Loan District. Average for the period 1918–1926.

ing values ranging from $50 to $100 an acre, the average increment in land value, exclusive of buildings per dollar increment in building value amounts to $0.56. On farms having building values ranging from $150 to $200 an acre, the average increment in land value amounts to $0.18 per dollar increment in building value while farms having building values ranging from $250 to $300 per acre have a decrement of $0.74 per dollar increment in building value.

These increments and decrements, it should be borne in mind, are differences in value, exclusive of building value and therefore represent the net difference after allowing for the cost of the additional building value. The curves in figure 25 are subject to changes over a period of time. It has been seen in figures 22 and 23 that the trend of appraised building values has been upward while appraised land values have not increased so rapidly. The ratio between land value and building value is therefore not constant. The ratios between land value and building value on 20-acre farms having $1000 to $1199 total building value are given in table 11. The trend in the ratio of land value to building value has decreased over the nine-year period, 1918 to 1926, from 4.68 to 4.06.

TABLE 10

AVERAGE APPRAISED VALUES OF LAND AND BUILDINGS FOR TWENTY-ACRE FARMS
COVERED BY FEDERAL FARM LOANS FOR DIFFERENT BUILDING
VALUES, 1918–1926

Building value class interval groups	Frequency	Building value, average	Land value, average	Value of buildings and land
		Dollars	*Dollars*	*Dollars*
1– 199	26*	112	3,897	4,010
200– 399	68	286	4,373	4,659
400– 599	104	479	4,297	4,776
600– 799	94	667	4,818	5,486
800– 999	90	848	4,686	5,535
1,000–1,199	134	1,031	4,688	5,719
1,200–1,399	71	1,251	4,634	5,885
1,400–1,599	76	1,473	4,912	6,385
1,600–1,799	46	1,658	5,216	6,874
1,800–1,999	36	1,763	5,164	6,927
2,000–2,199	93	2,023	5,144	7,167
2,200–2,399	31	2,270	5,207	7,477
2,400–2,599	46	2,486	5,267	7,753
2,600–2,799	28	2,654	5,671	8,325
2,800–2,999	19	2,867	5,389	8,256
3,000–3,199	41	3,041	5,236	8,277
3,200–3,399	13	3,231	6,124	9,355
3,400–3,599	30	3,472	5,683	9,155
3,600–3,799	15	3,667	5,015	8,682
3,800–3,999	13	3,870	5,168	9,038
4,000–4,199	23	4,023	5,702	9,725
4,200–4,399	12	4,258	5,355	9,613
4,400–4,599	9	4,469	6,673	11,142
4,600–4,799	9	4,667	5,072	9,739
4,800–4,999	4	4,900	6,336	11,236
5,000–5,199	15	5,043	5,757	10,800
5,200–5,399	6	5,308	5,941	11,249
5,400–5,599	3	5,433	4,722	10,155 ˊ
5,600–5,799	4	5,712	5,832	11,544
5,800–5,999	3	5,900	5,026	10,926

* Farms of zero building value are not included. This is to avoid confusion with farms for which building values are not given.

<div align="center">TABLE 11</div>

RATIO OF APPRAISED LAND VALUE TO BUILDING VALUE, 1918–1926, FOR TWENTY-
ACRE FARMS HAVING BUILDING VALUE RANGING FROM $1,000 TO $1,199

Year	Frequency	Total building value, average	Total land value, average	Ratio of land to buildings
		Dollars	*Dollars*	
1918	32	1040	4860	4 68
1919	23	1005	4780	4 20
1920	4	1000	4290	4 79
1921	4	1038	5280	5 1
1922	20	1031	5350	5 18
1923	12	1041	4170	4 0
1924	13	1060	4500	4 25
1925	10	1060	4113	3 88
1926	16	1045	4260	4 06

<div align="center">SALES PRICES OF DAIRY FARM LANDS</div>

Variables of the Problem.—The important variables causing differences in prices at which dairy farms are sold include productivity, value of buildings, size of farm, percentage of the farm irrigated, percentage of the farm in pasture and the character of community development. Many other causes of differences exist but either have a minor degree of importance or have not come within the scope of this study because of insufficient information. Effects of topography, poor drainage and irrigation, weeds, pests, alkali, hardpan and other physical defects have been excluded from this phase of the study also. Character of community development has been in evidence throughout as an important factor but field studies will be required to supplement the present analysis before proper evaluation can be made of this element of value. Irrigation costs, taxation and bonded indebtedness on lands come within the scope of this community factor. Although many of these disturbing elements are important, they have been excluded merely to provide a starting point.

The Heterogeneous Character of Dairy Farms.—Diverse methods of land utilization, diversity in sizes of farms, variations in the number of cows found on these farms, differences in the percentage of the total area irrigated and variable proportions in which different enterprises are combined are characteristics not only of farms in general but of dairy farms in particular. The dispersion is not so great, however, within such a group that we cannot discover general tend-

encies. We are continually compelled to decide whether accuracy will be increased or decreased by further limiting the scope because each limitation in scope reduces the number of observations and increases the disturbing effect of erratic measurements.

Deflation of Land Prices and Building Values.—Prices of land and buildings used are the total values declared by the purchaser before a notary when the resale was made divided by the farm acreage. This price per acre was divided by a deflation index calculated from our land resale price series described in the earlier pages. In computing this index, irregularities were removed by the use of a three times iterated three months moving average. The monthly averages thus computed were reduced to relatives using the average of the entire period, 1918–1926 inclusive, as a base. This index series includes the seasonal variations. At least one serious question arises in the use of such an index. The trends of land prices for different regions have been shown to be different. A series for each region can readily be calculated but within a region there is no certainty that a land price series for all kinds of farms is applicable to dairy farms and there are not sufficient dairy farms in the data used to make possible a dairy land price series for different regions. The only recourse was to use the relative index based upon price of all farms and test its effectiveness by results in correlation with and without the index. Coefficients of correlation were much improved by the use of the index. Most of the cases used in the constant productivity study were in the San Joaquin Valley. The land price deflation index for San Joaquin Valley is given in table 12.

In considering the deflation of building values, an attempt was made to study trends in building values. These have already been shown and discussed in connection with figure 22 on page 515. Although there have been quite radical changes in building prices since 1918, appraised values of buildings have certainly not followed these changes. The trend of building values per acre has been upward since 1918 probably due to added volume of buildings per acre. Appraisers and farmers have undoubtedly deflated costs to some extent but original costs have probably dominated appraisals. In the light of uncertainty as to what kind of index to use, building values have not been deflated in this study.

TABLE 12

LAND PRICE DEFLATION INDEX* BASED UPON A THREE-TIMES ITERATED THREE-
MONTHS MOVING AVERAGE OF SAN JOAQUIN VALLEY
RESALE PRICES, 1919–1927

Year	Month	Three-times iterated three months moving average	Index	Year	Month	Three-times iterated three months moving average	Index
1919	Jan	310	83.3	1923	Jan	345	92.7
	Feb	320	86.0		Feb	380	102.1
	March	330	88.6		March	410	110.1
	April	348	93.5		April	425	114.2
	May	370	99.4		May	425	114 2
	June	390	104.8		June	405	108.8
	July	390	104.8		July	370	99.4
	Aug	375	100 7		Aug	355	95.4
	Sept	360	96.7		Sept	350	94.0
	Oct	356	95.6		Oct	360	96.7
	Nov	362	97 2		Nov	375	100.7
	Dec	375	100.7		Dec	390	104 8
1920	Jan	380	102.1	1924	Jan	400	107.4
	Feb	400	107 4		Feb	405	108 8
	March	438	117.7		March	400	107.4
	April	480	128.9		April	395	106 1
	May	500	134 3		May	390	104.8
	June	485	130 3		June	380	102.1
	July	465	124.9		July	360	96 7
	Aug	455	122 2		Aug	335	90 0
	Sept	448	120.3		Sept	320	86.0
	Oct	425	114.2		Oct	315	84.6
	Nov	400	107.4		Nov	305	81.9
	Dec	388	104.2		Dec	290	77.9
1921	Jan	415	111.5	1925	Jan	275	73.9
	Feb	470	126 3		Feb	285	76.6
	March	470	126.3		March	305	81.9
	April	430	115.5		April	340	91.3
	May	374	100.5		May	375	100.7
	June	360	96.7		June	395	104.8
	July	360	96.7		July	415	111.5
	Aug	370	99.4		Aug	420	112.8
	Sept	380	102.1		Sept	395	104 8
	Oct	423	113.6		Oct	340	91 3
	Nov	475	127.6		Nov	300	80.6
	Dec	500	134.3		Dec	285	76 6
1922	Jan	535	143.7	1926	Jan	285	76.6
	Feb	574	174.2		Feb	285	76.6
	March	595	159.8		March	275	73.9
	April	580	155.8		April	275	73.9
	May	532	142 9		May	290	77.9
	June	500	134 3		June	300	80.6
	July	500	134.3		July	285	76.6
	Aug	515	138.3		Aug	255	68.5
	Sept	515	138.3		Sept	230	61.8
	Oct	470	126.3		Oct	230	61.8
	Nov	395	106.1		Nov	260	69.8
	Dec	345	92.7		Dec	285	76.6

* Index computed as relatives having a base period 1922–1926.

TABLE 12 (continued)

Year	Month	Three-times iterated three months moving average	Index
1927	Jan.........	290	77.9
	Feb....	285	76.6
	March..........	290	77.9
	April..................	295	79.2
	May.............	290	77.9
	June............	275	73.9
	July....	255	68.5
	Aug........	250	67.2
	Sept.......	245	65.8
	Oct........	240	64.5

Limiting the Problem to Three Variables—Size of Farm, Value of Buildings, and Price of Land and Buildings.—Economic and mathematical relationships between building value, size of farm, and land price introduce difficulties of analysis which must be clarified before broader studies of other land price elements can be made. Figures 26, 27, and 28 show relationships between size, appraised values of buildings and values of land and buildings on irrigated dairy farms having no pasture, having approximately equal productivity and having practically the entire area under irrigation. Although the productivity index does not enter here as a variable, it has served the very useful purpose of holding that factor constant. The farms have also been selected so as to be free from conditions of alkali, hardpan, poor drainage, or less than first class irrigation in order to reduce the number of variables and the amount of the dispersion. It is believed that the data so secured are valuable for the purpose of giving the characteristics of certain important relationships although actual quantitative measurement may be subject to slight change when more data have been collected for further analysis. Owing to the constant improvement of farms, the relationships between building values and land values are constantly changing, greater building values being associated with given land values as time goes on. This change, however, should not interfere with the fundamental characteristics shown in these charts.

We have already seen that as buildings are added to land, eventually a point is reached where the added building value ceases to add value to the land. The same tendency is in evidence in this analysis although the higher points on the curves are supported by meagre data. It is not frequently the case that a farm is over-developed in

regard to buildings, but among the data available many instances support the conclusion that the principle of proportionality operates with respect to the actual prices at which land changes hands. This characteristic is responsible for the shape of the curves in figure 26. For building values higher than those shown in figure 26, the plotted values became so scattered, curve fitting was impracticable.
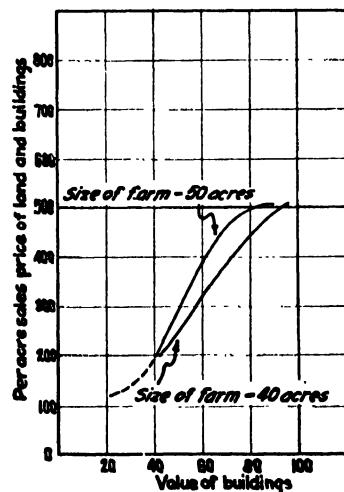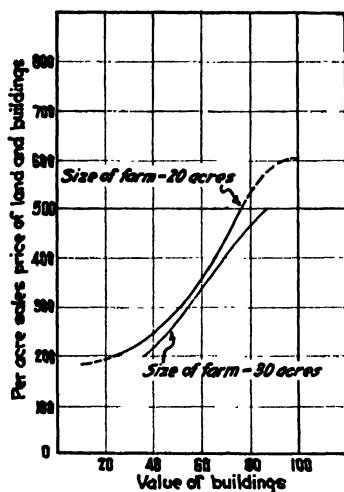
Fig. 26   Fig. 27

Fig. 26. Relationship between value of buildings and price of land and buildings for 20-, 30-, 40-, and 50-acre farms.
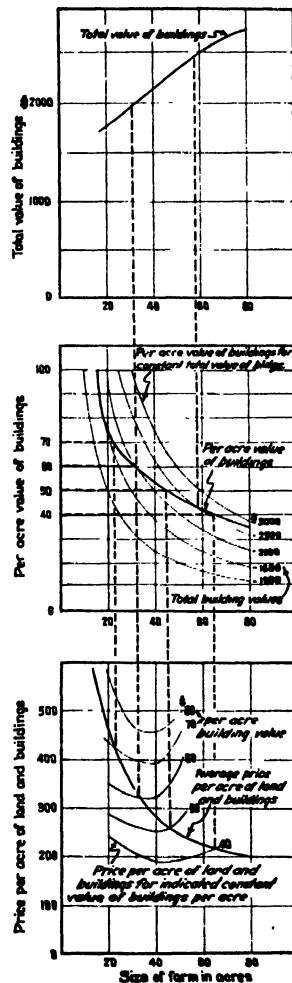
Fig. 27. Inter-relation between total and per acre building values, and price per acre of dairy farm lands for farms of different sizes.
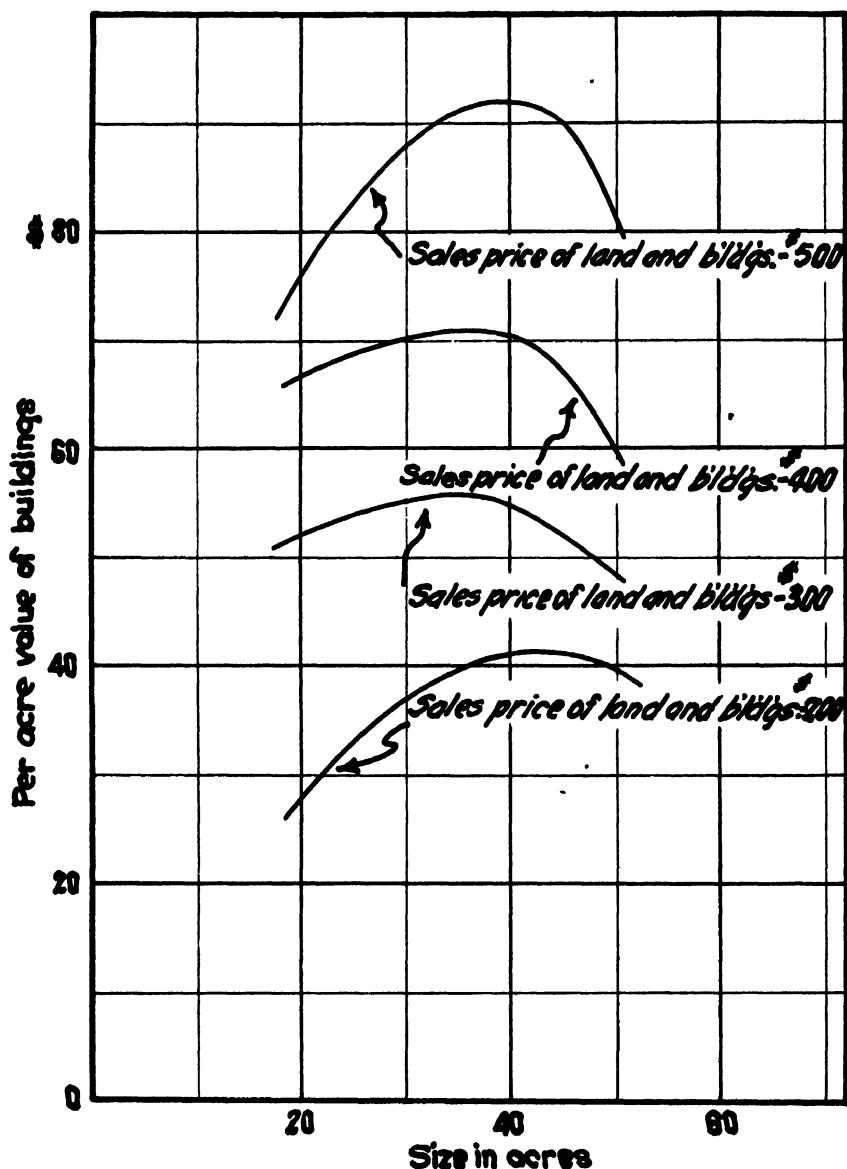
Fig. 28. When sales price per acre of land and buildings is held constant, the relation between per acre value of buildings and size of dairy farm in acres is expressed by a curve which, for size of farm between 20 and 50 acres, is convex upward. A given increment in the value of buildings is not accompanied by the same nor a proportional increment in value of land and buildings.

However, the scattered points indicate a downward tendency in the relationship between value of buildings and per acre sales price of land and buildings. It is natural that a few cases only could be obtained to illustrate this inefficient condition of farm improvement especially when the source of data is considered. Two 20-acre farms in the San Joaquin Valley will illustrate the tendency. Each of these farms is within three and one-half miles from town. Each is devoted to the production of field crops and dairy. The soils of these farms are sandy loam and fine sandy loam which have been shown in the preceding analysis to have approximately equal yield values. Temperature conditions are uniform with respect to each farm. Each has first class irrigation facilities. One of them has somewhat greater livestock development and has 18 acres in alfalfa while the other has 12 acres of alfalfa. The first of these farms has a per-acre building value amounting to $75, whereas the second has a building value of $217 per acre, a difference of $142 per acre in building value. The difference in deflated sales prices of these farms was $45, the farm having the higher building value corresponding to the lower sales price. We should guard, however, against drawing conclusions from individual sales of this kind. It is the general position of the scattered points in the different size groups studied which has lead to the conclusion that it is possible for value of land and buildings to be reduced by excessive addition to buildings. For most practical purposes land value studies will fall within the range of increasing value per acre of land and building for each increment in added building value, but the point may often be reached where increased building values ceases to add to the value of the land exclusive of buildings. The shape of the curve of relationship between value of buildings and sales price of land and buildings for 20-acre farms is quite different from the curve showing the same relationship on 50-acre farms. There are two reasons for this difference. In the first place, for economic reasons, diminishing returns to investment in buildings begin at a different point on 50-acre farms from that on 20-acre farms. In the second place, the purely arithmetical relation between total building values, size of farms, and building value per acre introduces the same difficulty of isolating the economic from the mathematical elements as was seen in the case where value of land and buildings in relation to size of farms was affected by these two different factors.

Inter-Relations Between Total and per Acre Building Values and Price per Acre of Dairy Farm Land for Different Farm Sizes.— Figure 27 is composed of three different sections each of which is

designed to bring out a special phase of the inter-relationship among these three variables. The upper section shows the simple relationship between average total appraised building value and farm size. The next section below this is identical with figure 24 previously described except it applies to dairy farms of the particular homogeneous group under discussion. This portion of the figure, together with the upper portion of the chart differentiates between the economic and mathematical variables, size of farm and value of buildings. The light curved lines are, as in figure 24, the rectangular hyperbolae giving the mathematical relation between size of farm and value of buildings per acre. The heavy curve in this central portion of the figure is the curve of relationship between size of farm and average value of buildings per acre. This curve is based upon the average building values given in table 13. This curve crosses each hyperbola at points vertically below the point on the total building value curve corresponding to the total building value represented by that hyperbola.

TABLE 13

AVERAGE PER ACRE SALES PRICES OF LAND AND BUILDINGS; APPRAISED VALUES OF
BUILDINGS AND AVERAGE SIZES OF FARMS FOR IRRIGATED DAIRY FARMS
NOT HAVING ORCHARDS NOR PASTURE FALLING WITHIN
DIFFERENT CLASS INTERVALS OF SIZE

Size class interval	Average size	Average appraised value of buildings	Average sales price of land
Acres	*Acres*	*Dollars*	*Dollars*
10–29	18.3	93	421
30–49	39.2	58	318
50–69	57 0	31	216
70–89	79.3	36	204

In the lower portion of the figure is a set of curves concave upwards showing the relation between size of farm and price of land and buildings for constant per acre building values. These curves in figure 27 have been derived from a set of curves similar to those in figure 26, and, together with them, have been derived from a smoothing of the raw data into a surface describing the relationship between the three variables under discussion. In this lower portion of the same figure is another curve showing the relation between size of farm and average price of land and buildings for average building values corresponding to the prices and sizes in each case. This curve is based

upon average prices of land and buildings given in table 13. In this curve, building value is a variable element and each point on the curve is the result of the average combination of size-building value and land price. The inter-relations of the different variables and the significance of the different portions of the illustrations are emphasized by the dotted lines connecting the different parts of the diagram. The point where the curve of total building value in the upper portion of the figure crosses the $2000 building value line is vertically above the point where the curve showing the average relationship between building value and size of farm crosses the curve of $2000 constant total building value. The point where the average building value curve in the central part of the figure crosses the line of $50 per acre building value is vertically above the points where the curve express-ing the relationship between size of farm and average price per acre for land and buildings crosses the curve of relationship between size of farm and price of land and buildings for a constant building value of $50. Referring again to figure 26 with the new relationships revealed in figure 27 still in mind, the inter-relationships of size-building value and price of land and buildings may be better under-stood. Between price for land and buildings of $200 to $500 per acre, 30- and 40-acre farms have approximately $6 difference in price of land and buildings associated with each $1 difference in value of buildings. The 20-acre farms and 50-acre farms are more curvilinear between these prices and have differences in price of land and build-ings, ranging for the price range given, from $3.50 to $10 for a differ-ence of $1 in building value, the lower increments in price being for lower priced farms in the case of the 20-acre size and for the higher priced land in the case of the 50-acre farms. For a constant per acre building value, farms smaller than 35 acres generally have increments in price of land and buildings, for each acre difference in size, which are rapidly increasing as the size of farm decreases. For farms greater than 40 acres, increments in price of land and buildings for each acre difference in size rapidly increase up to certain limits, as size increases. But this latter tendency is due largely to the increasing importance of a given constant building value per acre as the size of the farm increases. The lower portion of figure 27 illustrates these tendencies.

Similar reasoning may be brought to bear in explaining the curves in figure 28 which show the relations existing between size of farm and per acre value of buildings for constant values of land and build-ings. This figure is also a result of the same surface of relationship

among the three variables. The curve showing the average relationship between size of farm and price per acre of land and buildings now has greater significance. It owes its shape to the mathematical hyperbolae first shown in figure 24, to the economic factors which cause lower per acre building values to be associated with larger farms and to the many reasons enumerated above for large farm sizes to be associated with lower per acre values of land exclusive of buildings.

TABLE 14

ESTIMATED VALUE OF BUILDINGS AND ESTIMATED SALES PRICES OF LAND AND
BUILDING COMPRISING IRRIGATED DAIRY FARMS NOT HAVING ORCHARDS
NOR PASTURE AND HAVING APPROXIMATELY EQUAL PRODUCTIVITY*

Size	Estimated average per acre value of buildings	Estimated average per acre price of land and buildings
	Dollars	*Dollars*
20	74	470
30	62	340
40	54	280
50	47	245
60	42	225
70	38	205
80	34	195

* Table 14 has been compiled from figure 27.

The Effect of Introducing Other Variables. — The foregoing analysis indicates that the introduction of additional variables will make necessary extreme caution if reliable results are to be obtained. No attempt will be made in the present discussion therefore to set up the partial correlations for additional variables. The simple correlations between land price as a dependent variable and productivity, per cent of farm irrigated, per cent of farm in pasture, building values, and size of farm as independent variables when all of these are present with interacting influences upon each other are shown in figures 29 to 33 inclusive. The correlations are between median values of class interval groups given in table 15. These graphs should be used with careful judgment. The size-land price curve, figure 29, is distorted especially in the case of larger farms by the presence of the other variables. Building values bear a different relation to price of land and buildings. The lower building values in figure 30 are for the larger farms having more or less pasture. In the case of higher building values and higher land values there is closer correspondence between the curves constructed under the two different sets of condi-

tions. This is because the farms in this portion of the curve are predominantly those comprising the data from which the other curves were drawn. In other words, high building values and high land values are associated with irrigated farms without pasture. The other relationships shown in figures 31, 32 and 33 are subject to the same complications.
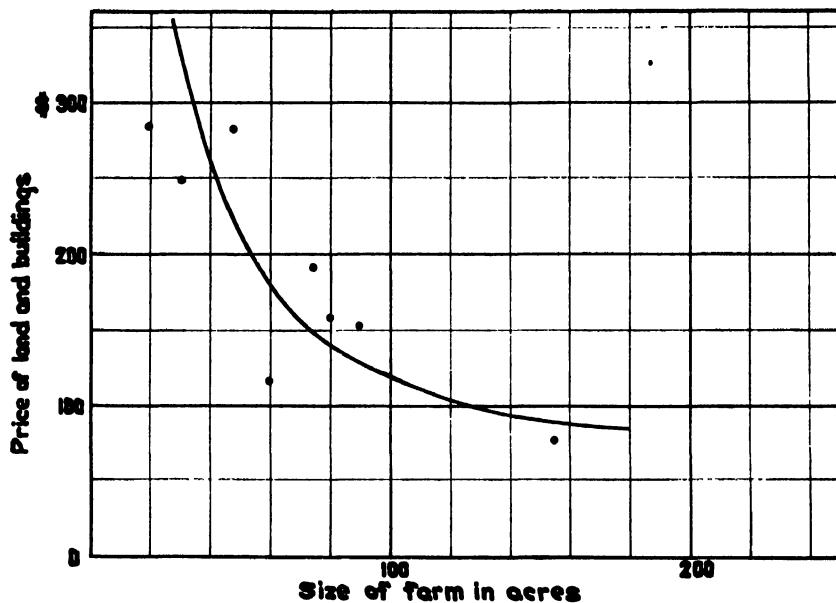
Fig. 29. Figures 29 to 33 inclusive show correlations between price of dairy farm lands and various other factors. Figure 29 shows the relation between size of dairy farm and price per acre. It should be borne in mind that these are not partial correlations and each scatter diagram is influenced by the interactions of all of the factors. Each of the points plotted in these illustrations is the median value of a class interval group. The number of cases in each group, however, is small, and the curves are presented as approximations only.

An important use which this set of charts may serve is that from them a typical farm may be set up showing the average conditions which may be expected to prevail with regard to any particular price of land and buildings. This may be used as an approximate guide when proceeding according to ordinary methods of appraisal. Such a guide would be much more satisfactory than the usual scattered and unorganized sales price data. For this purpose, table 16 has been prepared showing such typical combinations of conditions for different prices of land and buildings. It must still be borne in mind that there is a much larger group of farms subject to variable conditions of drainage, irrigation, hardpan, etc., which has not been represented.
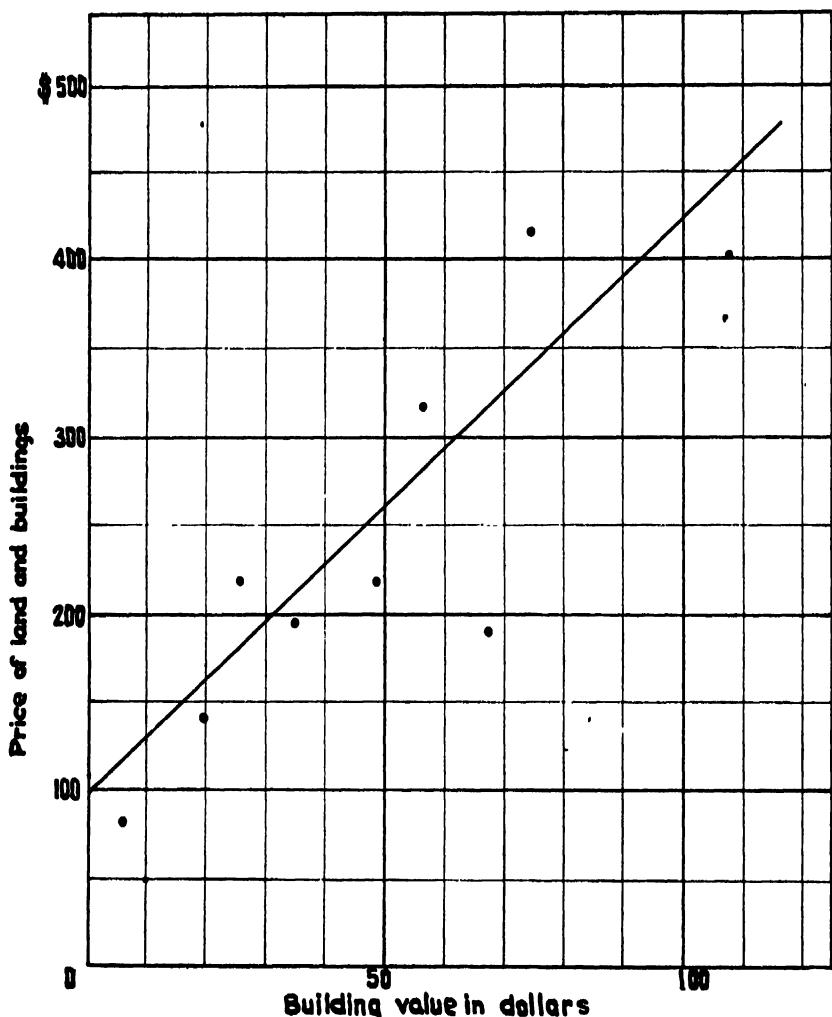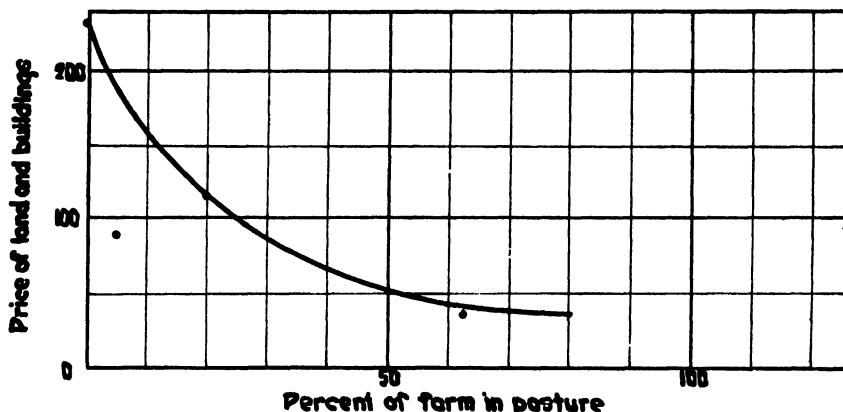
Fig. 30. Relation of building value to price per acre of dairy farm land.
The method of construction and precautions to be taken in the interpretation
of this illustration are the same as for figure 29.
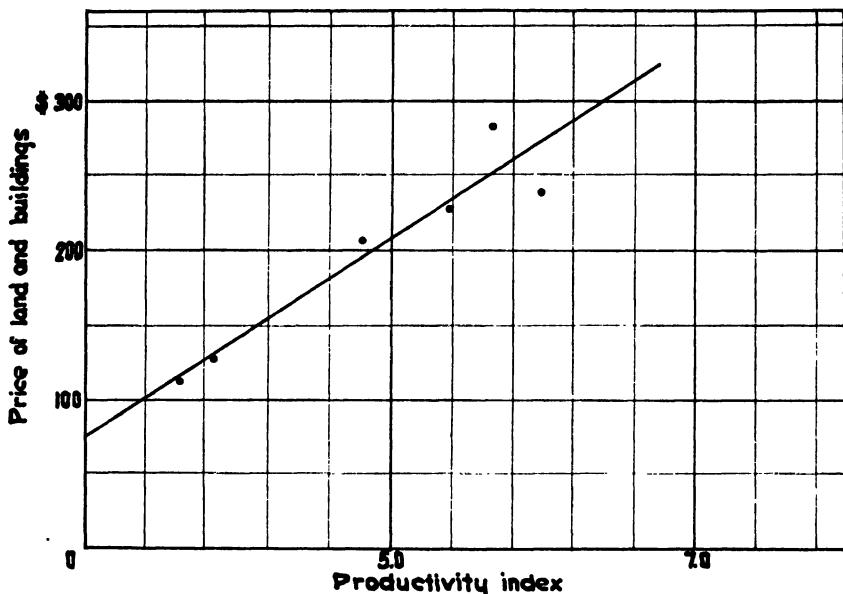
Fig. 31. Relation of productivity index to price per acre of dairy farm lands. The method of construction and precautions to be taken in the interpretation of this illustration are the same as for figure 29.
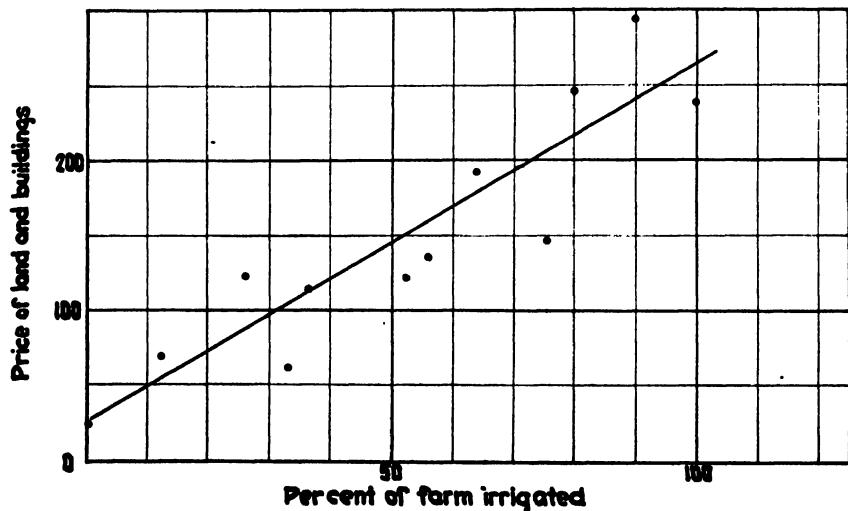
Fig. 32. Relation of per cent of irrigated land on each farm to dairy farm land price. The method of construction and precautions to be taken in the interpretation of this illustration are the same as for figure 29.

The well-defined correlation between price of land and buildings and productivity is gratifying after the tedious process of constructing this index. Although its significance is mingled with the effect of other variables, in figure 31, the very important fact is brought out that a given average productivity is associated quite definitely with a given average price of land and buildings not withstanding that there are many elements contributing to the value represented in that price.

Fig. 33. Relation of per cent of farm in pasture to price of dairy farm land. The method of construction and precautions to be taken in the interpretation of this illustration are the same as for figure 29.

TABLE 15

Median Deflated per Acre Prices of Farm Real Estate for Different Class Intervals of Building Value, Size, Per Cent of Farm Irrigated, Productivity and Per Cent of Farm in Pasture

Building value		Size		Per cent irrigated		Productivity index		Per cent of pasture	
Class interval	Corre-sponding median price	Class interval	Corre-sponding median price	Class interval	Corre-sponding median price	Class interval	Corre-sponding median price	Class interval	Corre-sponding median price
0–9	80	10–19	412	0–9	24	3.4–3.79	112	0	243
10–19	139	20–29	284	10–19	68	3.8–4.19	127	1–9	95
20–29	218	30–39	246	20–29	123	4.2–4.59	315	10–19	170
30–39	193	40–49	281	30–39	61	4.6–4.99	206	20–29	118
40–49	217	50–59	—	40–49	114	5.0–5.39	226	over 29	39
50–59	313	60–69	125	50–59	122	5.4–5.79	282		
60–69	190	70–79	191	60–69	192	5.8–6.19	248		
70–79	440	80–89	157	70–79	150				
80–109	394	90–99	150	80–89	246				
over 109	492	100–169	76	90–99	294				
		over 169	32	100–	238				

<div align="center">

STATISTICAL METHOD

</div>

The results of this research have not been reached without trial and error in the use of a number of different methods of statistical analysis. The machine sorting process and mechanical tabulation which served so admirably in the development of the productivity index and in orienting the research had to give way to a detailed analysis of individual farms in the final land price study. More than 15,000 cards were punched in code giving scores of descriptive items for each farm studied. The rapid sorting of these into classified groups and the tabulation and totaling of yield data gave the basis of the averages used in estimating alfalfa yields for the different temperature stations and for the different soil textures.

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TABLE 16

AVERAGE COMBINATIONS OF ESTIMATED PRICE PER ACRE, SIZE, PRODUCTIVITY INDEX,
PER CENT OF FARM IRRIGATED, PER CENT IN PASTURE, AND BUILDING
VALUE PER ACRE FOR DAIRY FARMS IN THE ELEVENTH
FEDERAL FARM LOAN DISTRICT

</div>

Price per acre	$100	$125	$150	$175	$200	$225	$250	$275	$300
Size, acres....	127	95	72	60	52	46	41	36	35
Productivity	3.4	3.8	4 2	4.5	4.9	5.3	5.6	6 0	6.4
Per cent irrigated . .	31	42	52	63	73	84	94	100	100
Per cent pasture...... ...	26	18	11	6	3	1	0	0	0
Building value, dollars ..	0	8	16	23	31	39	48	54	62

In making the detailed study of resale prices, the description of each farm was recorded on strip cards and the process became one of careful manual sorting and tabulating in place of the machine sorting and tabulation in the earlier period of the investigation. This was made possible by the smaller numbers of cases handled. Not only has it been necessary to give much attention to the method used in selecting and tabulating the data but methods of analysis of time series and of price differentials have required much time and laborious effort.

Inadequacy of Multiple Correlation in Land Price Analysis.—This study as well as many previous studies indicates that economic relationships are not simple straight line correlations, but rather of a complicated and varied curvilinear character. The ordinary curvilinear multiple correlation carried out by the solution of normal equations adjusted for curvilinearity by successive approximations is limited in its application to the complex problems of land valuation.

Curvilinear multiple correlation, as ordinarily carried out, does not give proper significance to the effect of combinations of other factors. This is probably its chief limitation. The effect of a given independent variable upon the dependent variable shows a different curve under changed conditions of other variables. In fact it has very definitely been found that any given regression line changes in shape, if curvilinear, and slope for changing conditions of other variables. When one conceives the problem to be of such a nature, it no longer is a study of parallel curves in a plane which is the assumption of curvilinear multiple correlation, but rather a warped surface. In fact the problem must be set up as a group of such surfaces. Such studies require more sampling technique than is usually applied. Enough data must be collected to properly represent different portions of the various surfaces. If this is done completely, multiple correlation gives way to a number of related simple correlations followed by the determination of relationships between the various simple regression lines or surfaces. There is a limit to the extent to which this can be carried out, not only because of the cost involved but because of the non-existence of farms in certain classifications. Increase in numbers of cases is sure to introduce new variables.

The importance of this method of analysis was emphasized in the soil-temperature analysis. It may be noticed by referring to figure 17 and the discussion thereabouts that for different mean temperatures, variations in range of temperature have entirely different effects—in fact opposite effects. An increase in range of temperature shows a positive increase in yield for the lower mean temperatures and a negative relationship for the higher temperatures. Had the ordinary method of multiple correlation been applied, the average effect of differential in temperature range upon yield would have appeared unchanged irrespective of the mean temperature, whereas the chart shows that the relationship is very different under different temperature conditions.

Another example indicating the inadequacies of multiple correlation in such studies is illustrated by the study of the interrelations of resale price, building value and size of farm. In fact, as we have seen, most of the elements of land value have curvilinear relationships which follow the characteristic curve of diminishing returns. These curves for different sizes of farms cross and recross each other.

Methods Used.—The only possibility of following and isolating such curvilinear relationships is to study each group separately by selecting samples and eliminating many of the variables by sorting.

The present study, employed with some success, the use of mathematical formulae in bridging gaps and in fitting surfaces to some of the grouped data. The three variables just mentioned, size of farm, building value, and price of land and buildings yield to manipulation with the formula $y = a + b \dfrac{2}{(e^{K(x-c)} + e^{-K(x-c)})}$ in which y is the price of land and buildings, x is the value of buildings, while a, b and c are functions of the size factor and K is a constant which gives flexibility to the shape of the curve. Any section of the surface obtained by constant values of a, b, and c is similar in shape to the normal curve of error. This shape is also characteristic of many of the curves following the principle of diminishing returns. Where the data do not follow a bisymmetrical curve, a "skew" can be obtained in the above formula by the use of $f(x)$ in place of x. The actual curves shown in figures 26, 27, and 28, however have been developed by reducing the problem to three variables by sorting into groups and by plotting curves between two of these variables holding the third constant. Overlapping class interval groups were used, making possible a large number of such curves for different average values of the third variable. When these curves were completed, the variable which was held constant in the first place was used as a variable in relation with one of the others and cross section curves were constructed using values estimated from the previously constructed set of curves. Smoothing was thus carried out in two directions at right angles to each other and a curved surface was thus constructed.

Complications of Additional Variables.—The simple correlations presented in figures 29, 30, 31, 32 and 33 represent a work only partially completed, it is true, but useful if the limitations are kept clearly in mind. It would not be difficult to set up a multiple correlation equation of regression for estimating land price from these variables. It is believed, however, that more careful study should be given to correlation methods to be used in completing this analysis before it is safe to present such an equation.

The Adequacy of the Frisch Method of Time Series Analysis.—The method of time series correlations used in this investigation has been somewhat of an experiment in which the adequacy of the Frisch method of handling cyclical problems has been tested. This method has been studied having in mind the following prerequisites of an adequate time series analysis. The method should have inherent within it the possibility of studying (1) cycles and trends of various

orders; (2) changing slopes in trend; (3) changing lengths of the several cycle periods; and (4) changing amplitude of oscillations. It has been with the desire to observe these four aspects of the problem that this comparatively new treatment of time series has been applied in the present study.

Lack of flexibility due to mathematical assumptions and formulae has characterized most of the current methods. In the present and previous studies, it has been found that even long time trend analyses are not adequately handled by the straight line or parabolic methods. The concept although quite different from other methods is simple. Instead of fitting a secular trend line and then removing seasonal variations, seasonal and erratic variations are removed in the process of fitting the first trend. The raw data are smoothed, and points of inflection in the series are located and connected by a smooth curve. The inflection points of the resulting graphs are located and connected by a second smooth curve and so on indefinitely. Cycles of various orders are thus isolated and their deviations from trends of succeeding orders form the basis of the analysis. Although it is not without limitations and difficulties of application, the advantages of the Frisch method are numerous. Flexibility in meeting the complicated cyclical combinations of a series has proved to be an advantage in its favor. Comparison with results obtained by the use of a trend line fitted by the method of least squares and ordinary means of removing seasonal variation shows that although the cyclical characteristics described by the older methods were different, the long time trends were similar. Relationships between different time series has been discovered by this method which would have passed unnoticed in the use of former methods. It more adequately meets the problem of changing seasonal variation and more completely removes erratic influences leaving a well defined cycle from which disturbing elements have been removed. The result is that the coefficient of correlation more nearly describes the true relationship between the cycles of the different series.

The trend developed is not an "average" trend in the sense that the sum of the deviations from it is zero. In fact, when applied to pig iron production in the United States it has been found that more of the values fall above the line of trend than below it, indicating that prosperous years have been more numerous than years of depression. This tendency cannot be said to be a defect. It is merely a characteristic that must be recognized. A very important advantage of the method is that it yields to graphical analysis and much time is saved in making trial correlations when search is being made for related

series. The method is subject to two defects which can be eliminated by the acquirement of practical judgment in its use and by the maintenance of strict honesty on the part of the analyst. Certain erratic fluctuations in the data may introduce minor cycles which if adherence to the method were maintained would make it possible for these erratic variations to take the place of seasonal or other cyclical variations. As will be seen when the method is explained more fully, this may result in the confusion of cycles of one order with those of another.

The second difficulty arises in drawing in the trend lines established by the location of the inflection points. These points falling at some distance from each other are connected by smooth curves. The curvature between inflection points allows the judgment of the analyst to enter and introduces a personal element in the exact location. The general position and shape of the line, however, is fixed by the statistics. Balancing the advantages against the disadvantages it seems safe to conclude that the method deserves more general use by American analysts.

Description of the Method.—The underlying assumption in this method is that an economic time series is a composite curve of many components, or trends of several orders, each cyclical in nature and fluctuating about a trend of higher order. In the process of isolating these several trends, this method first eliminates trends of the lower orders (usually the seasonal variation) leaving to be isolated the trends of higher orders. Dr. Frisch has developed two methods for the solution of the complex problem of isolating the different cyclical trends of a series. One of these he calls "the method of normal points," the other, "the method of moving differences." Each is based upon the construction of a curve of second differences. The first of these methods, that is, the normal point method, is based upon the fact that the cyclical fluctuation of the curve obtained by plotting the original data passes its "normal" at the same point where the second difference curve becomes zero. The second method, that is, the method of moving differences, depends upon the fact that within certain limiting conditions the cyclical fluctuation of the composite curve formed by plotting the original data is proportional to the ordinates of the second difference curve, the constant required to reduce the ordinates of the second difference curve to those of the curve showing cyclical fluctuations being a function of the distance between zero points, that is, between the normal points determined by the first method. The method of moving differences has not been tested in the present

study.   The analyses of this investigation have been based entirely
upon the normal point method.   There are certain limiting conditions
which must be observed in connection with this method.   One of
these limiting conditions is that, for accuracy, the ratio between the
number of cycles in a trend of a given order to the number of cycles
in the same length of time in the trend of the next higher order should
be fairly high.   If the period length in any given trend is approxi-
mately the same or only slightly less than that of the next succeeding
higher order, an error is introduced due to the curvature in the trend
of high order.

In the discussion which follows, no attempt is made to go into the
intricacies of the two methods mentioned above.   Only a brief descrip-
tion of the method of normal points is given.   The application of the
method is very simple.   Observance of the limiting conditions, a dis-
cussion of which must necessarily be too elaborate for inclusion here,
involves certain tests which are based upon difficult mathematical
calculations but which in themselves are not elaborate.

In order to explain the differential aspect of the method, it may
be well to bear in mind a simple concept of a smoothly fluctuating
series.   Zero points of the second difference curve are points of inflec-
tion on the original curve.   These points of inflection determine the
position of the first trend line from which deviations of the points on
the original curve constitute the cycle of the lowest order.   It is upon
this basic analysis that the entire system of trend eliminations is
based.   Having obtained trends of successive orders, points of inflec-
tion are spotted, and new trend lines drawn through the cyclical
deviations studied from these higher trends.

The actual procedure followed in the case of the study of cycles in
land prices was first to plot the monthly average prices against time
as is shown by the dotted line in figure 4.   Because of the irregular
fluctuations of the curve, it was necessary to compute a twice iterated
three-months moving average (the moving average of the moving
average).   This smoothed curve was obtained readily by means of
graphically locating the points rather than working out the averages
by arithmetic calculations.   The method of graphical construction of
moving average as used in this study is that developed by Ruth
McChesney.[32]   Upon the completion of the twice iterated three-months
moving average, the points of inflection were located by graphical
analysis at a point where a three-times iterated average crossed the

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[32] McChesney, Ruth.   Graphical construction of moving averages.   Jour.
American Stat. Assoc. 23[(162)]:164–172.   June, 1928.

twice iterated moving average. Through these inflection points was drawn a smooth line, designated as the *trend of first order*, to form the basis of measuring the fluctuations of the cycles of first order, which in this case were usually those of seasonal variations.

The inflection points of the trend of first order were located and connected by means of the smoothest line possible. This line is called the *trend of second order*. The difference between the trend of first order and the trend of second order brings out the cyclical variations of the second order. If these second trends be reduced to a horizontal base, cycles of different series may be compared as is common in studies based upon other methods of analysis. The above procedure may be carried on indefinitely, limited only by the length of period for which data are available, resulting in trends and variations of several orders. Most of the comparisons in the land price study were made with respect to the deviations of the first from the second trend; i.e., the cycles of the second order, the cycles of the first order being eratic and seasonal variations.

CONCLUSION

A conclusion to such a study must necessarily be brief, for to present the findings in any detail would lead at once into the intricate complications which have already been presented. If a step forward has been made, it is in the the direction of method of approach to this most difficult problem. The relation of the principle of proportionality to scientific appraisal has probably been more definitely established. The classification of productive elements together with determinations of the effect of these elements combined in different proportions may replace, for many purposes, the classification of land itself. The numbers of possible classes of farms is almost infinite while the important elements are capable of finite description. The dynamic factors affecting land price through their effect upon economic supply of and demand for land gives us an insight into the changing importance of income in relation to land valuation, and may be a convincing argument that income, while it is the basis of value, does not have a constant relationship to value. This does not mean that sales price analyses should entirely replace income as a measure of value. We need all the information possible regarding income and sales prices. Finally, by the analysis of different elements of value in different combinations the way has been suggested, in fact

demonstrated, by means of which we may be able to construct methods of measuring probable producing power—not on the basis alone of what the land is producing but on the basis of what it is capable of producing. In light of its being more or less a pioneering effort, the findings so far presented, though meagre have justified the tedious process of isolating these few facts and principles from the great mass of data in which they were found.

YELLOWS DISEASE OF CELERY, LETTUCE, AND OTHER PLANTS, TRANSMITTED BY CICADULA SEXNOTATA (FALL.)

HENRY H. P. SEVERIN[1]

INTRODUCTION

Celery affected with yellows was first observed in the San Joaquin delta in 1925 during an investigation of economic plants naturally infected with curly top. During 1926 and 1927 an average of 5 per cent of the crop was affected in the delta, the most important celery producing region in California; in 1928 about 10 per cent of the celery showed symptoms of the disease. Some celery growers hoe out the diseased celery so that an exact percentage of yellows could not be obtained in some fields.

During 1927, 7,000 acres of celery, valued at $3,096,310, were grown in the San Joaquin delta so that the loss due to yellows amounted to $154,815. During 1928, 7,400 acres of celery are being grown in the delta, but the value of the crop has not been estimated; the loss due to this disease will probably exceed $300,000.

Lettuce yellows, known as white-heart or rabbit-ear in New York, and Rio Grande disease in Texas, is a rather serious malady of lettuce. Lettuce yellows is of no economic importance at present in California, but its nature is such as to demand field observations year after year to determine its potential importance.

According to Kunkel[4] aster yellows "is so serious in many sections of the country that the planting of asters is being restricted or

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even abandoned. Aster plots showing 90 to 95 per cent of yellowed plants are not uncommon throughout the eastern United States."

Aster yellows first made its appearance in California during 1925 and in the next three years the disease has spread rapidly through the middle and southern sections of the state. Yellows of flowering plants is already causing some concern to seed and flower growers in certain localities.

An investigation was undertaken to determine whether insects, especially leafhoppers, transmitted this disease to celery. Experiments were conducted to determine the relation of the celery disease to aster and lettuce yellows. The relation of yellows to curly top of sugar beets was also investigated. The characteristics, distribution, flights, food plants, and overwintering stage of the six-spotted leafhopper are discussed in this paper.

NAME OF THE DISEASE

A large number of plant diseases are designated by the term "yellows" some of which belong to the virus diseases, as does the one under consideration, while others are caused by fungus parasites. Kunkel[5] has experimentally transmitted aster yellows with the six-spotted leafhopper, *Cicadula sexnotata* (Fall.) to more than seventy species of plants in twenty-eight families. Celery yellows has been proven to be identical with aster yellows and should be classified with this disease.

"Yellows" of celery caused by a species of *Fusarium* occurs in Michigan, Indiana, Ohio, Pennsylvania, New York, Massachusetts, Connecticut, and New Jersey but has not been reported from California.

OCCURRENCE OF YELLOWS IN CALIFORNIA

Methods of Introduction into California.—The six-spotted leafhopper, *Cicadula sexnotata* (Fall.) has been known to occur in California for a long time, but the yellows disease which it transmits is a recent introduction into the state. Yellows disease may have been introduced into California through shipments of diseased perennial flowering plants from the Middlewest or East, or through cut flowers brought from the Middlewest or East by the traveling public. Successive migrations of infective six-spotted leafhoppers from the Middlewest to California appear to be entirely out of consideration

as a method of introducing yellows. According to Linford[6], aster and celery yellows first made its appearance in Utah during 1927. If successive westward migrations of infected leafhoppers had occurred the disease should have made its appearance in Utah first.

Distribution.—In California, celery yellows is known to occur in San Joaquin, Santa Clara and Monterey counties. No attempt has been made to determine whether this disease occurs in the celery districts of Santa Barbara, Los Angeles, Orange, and San Diego counties.

Lettuce yellows occurs in the Santa Clara and Salinas valleys and has been observed by Mr. Ivan C. Jagger, in his experimental summer plantings of letture at Chula Vista.

Smith, who worked with aster yellows in Massachusetts,[11] was first to notice aster yellows at Boulder Creek, Santa Cruz County during 1925; at Berkeley, Alameda County during 1926; and at Arroyo Grande, San Luis Obispo County during 1927. Many shipments of flowering plants, especially asters and zinnias, have been received and proved to be naturally infected with yellows, showing that the present known distribution of this disease is from Sonoma to Los Angeles counties.

PRODUCTION OF NON-INFECTIVE *CICADULA SEXNOTATA* (FALL.)

Kunkel[4] found that wheat and rye are immune to asters yellows and that the disease is not transmitted through the egg of *Cicadula sexnotata.*

In order to obtain non-infective *Cicadula sexnotata* for experimental purposes, adults captured on celery in the field were confined in cages enclosing wheat. After the nymphs hatched from eggs deposited in the wheat, all of the adults were removed from the cages. After the nymphs acquired the winged stage, the males were transferred from wheat to celery, asters, lettuce, and buckwheat. They failed to transmit yellows to these cultivated plants.

A low population of the six-spotted leafhopper was obtained on wheat owing to mildew, and hence Sacramento barley, which is resistant to mildew, was used in subsequent experiments. The six-spotted leafhoppers were collected in the grain field and foothills of the San Joaquin Valley and allowed to oviposit in barley. The insects which developed on barley were not able to transmit yellows.

CELERY YELLOWS

Symptoms.—The first symptoms to appear in celery infected with yellows by the six-spotted leafhopper in the greenhouse is a vertical or upright position of the petioles, which are somewhat longer than those of the healthy leaves of the same age. The petioles of the inner-most or youngest leaves are shortened and chlorotic and begin to twist (fig. 1) and intertwine (figs. 2, 3). Celery naturally infected with yellows sometimes shows a circular twist of the petioles (fig. 4). A general yellowing of the plant then develops with a premature blanching of the outer leaves. The vertical or upright petioles grad-ually assume a flat position. The petioles are brittle, break easily, and often crack (fig. 5). In the later stages of the disease, the heart of the plant decays (fig. 6), forming a soft yellowish brown rot which extends down into the base of the plant.

Incubation Period.—The incubation period of the disease was determined in eighty-seven Golden Self-Blanching celery plants infected with yellows. The six-spotted leafhoppers were fed for a period of two to three weeks or longer on diseased celery or asters removed from the field or on celery or asters experimentally infected with yellows in the greenhouse and were then transferred to healthy celery. Males were used rather than females in order to prevent oviposition. The length of time that elapses from the incculation of the plant until the petioles began to twist was considered as the incu-bation period of the disease. The minimum, maximum, and mean incubation periods during the four seasons of the year are given in tables 1 and 2. Celery plants used as a check or control remained healthy.

TABLE 1

INCUBATION PERIOD OF YELLOWS DISEASE IN CELERY INFECTED DURING AUTUMN AND WINTER

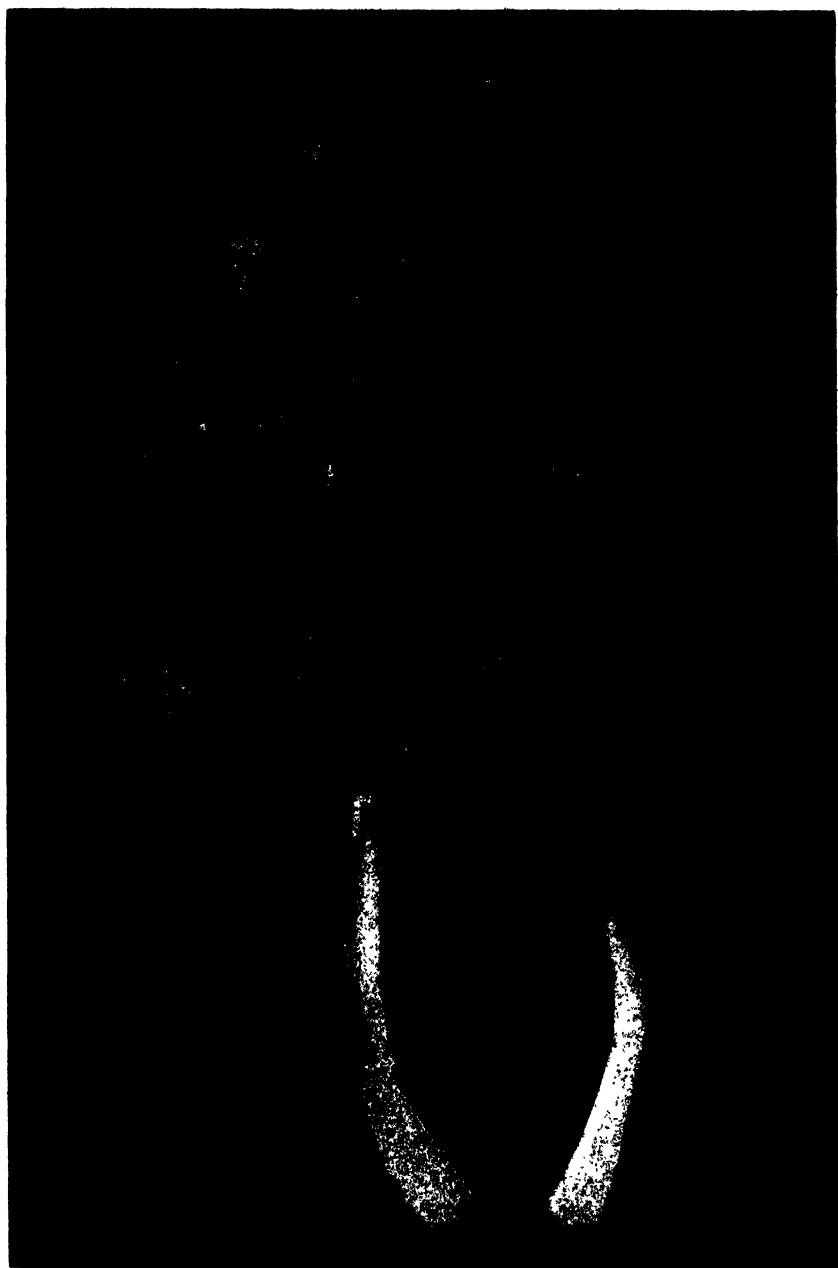| Dates leaf hoppers inoculated plants	Number of celery plants inocu-lated	Number of leaf-hoppers on each plant	Number of plants infected	Number of plants healthy	Minimum incubation period in plant *days*	Maximum incubation period in plant *days*	Mean incubation period in plant *days*
Sept. 26–Oct. 6	1	13	1	0	41	41	41.0
Nov. 29–Dec. 11	6	2	5	1	43	87	66.0
Dec. 9–17	9	3	5	4	38	53	43.8
Dec. 19–29	10	4	6	4	32	57	47 1
Dec. 10–20	4	3	3	1	97*	112	102.0
Dec. 11–21	2	3	2	0	99*	111	105.0
Jan. 3–20	5	10	4	1	30	60	42.2
Jan. 21–Feb. 4	5	6	5	0	34	60	50.0
Total	42		31	11			

* Incubation period in field.

Fig. 1. Celery (*Apium graveolens dulce*) leaves from a plant infected with yellows in the greenhouse, showing curved petioles.

Fig. 2. Celery (*Apium graveolens dulce*) plant naturally infected with yellows, showing twisted and intertwined petioles.

Fig. 3. Celery (*Apium graveolens dulce*) plant inoculated with yellows by infective six-spotted leafhoppers in the greenhouse, showing twisted and intertwined petioles.
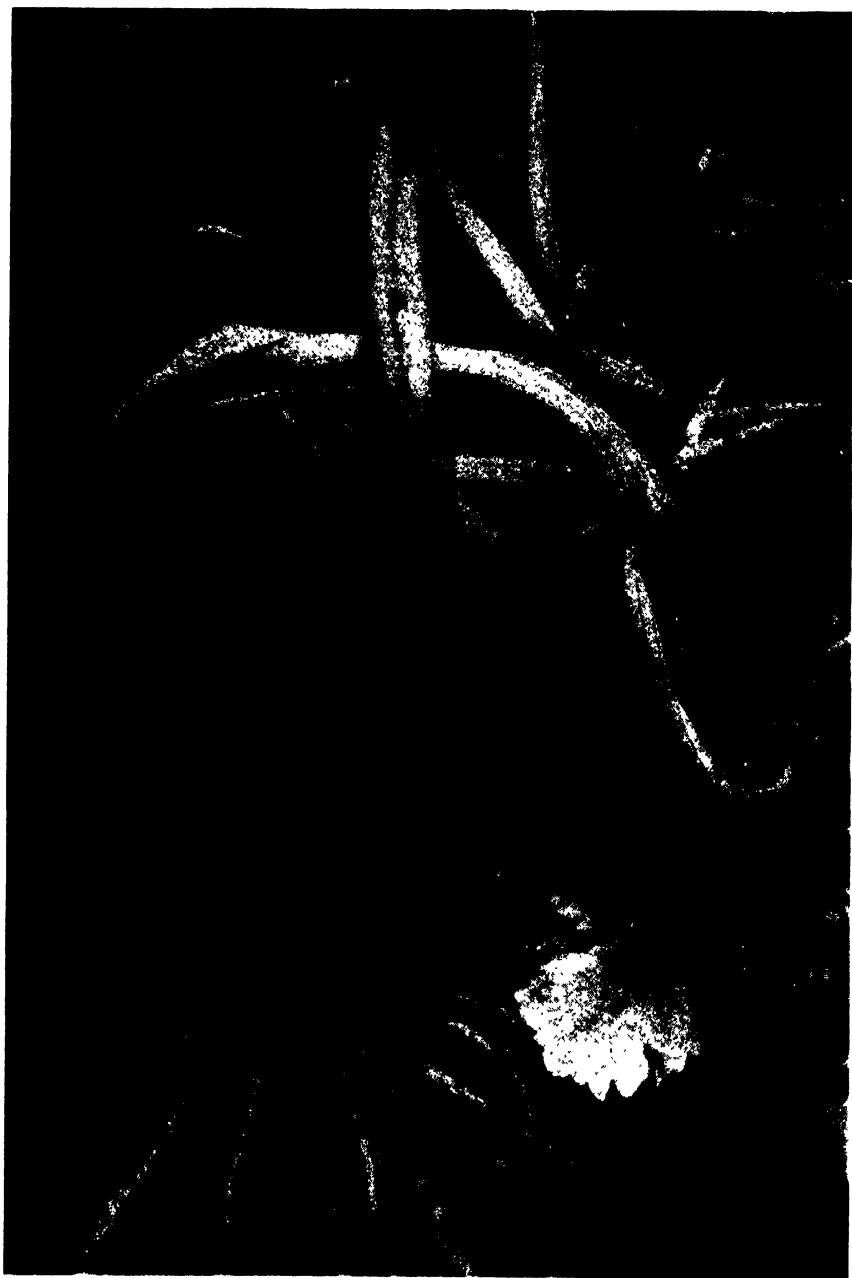
Fig. 4. Celery (*Apium graveolens dulce*) plant naturally infected with yellows, showing circular twisting of petioles.

Fig. 5. Petioles of celery (*Apium graveolens dulce*) plant naturally infected with yellows, showing splitting.

Fig. 6. Longitudinal section of celery (*Apium graveolens dulce*) plant naturally infected with yellows, showing decay of heart leaves.

TABLE 2

INCUBATION PERIOD OF YELLOWS DISEASE IN CELERY INFECTED DURING SPRING
AND SUMMER

Dates leaf hoppers inoculated plants	Number of celery plants inoculated	Number of leaf-hoppers on each plant	Number of plants infected	Number of plants healthy	Minimum incubation period in plant *days*	Maximum incubation period in plant *days*	Mean incubation period in plant *days*
Apr. 25–May 8 . .	11*	5	10	1	26	63	47 6
May 29–June 12 .	12	25	11	1	27	59	39 1
June 12–19.... .	12	20	8	4	24	34	27 3
June 25–27	12	20	9	3	18	32	22 7
June 27–July 9 .	12	20	8	4	29	50	36 7
July 21–28 ..	12	20	10	2	44	79	56 8
Total .	71		56	15			

* 11 plants that remained healthy in the incubation trials reported in table 1, inoculated a second time.

It is evident from tables 1 and 2, that the shortest incubation periods occurred in celery infected in late May and during June. All celery infected during May and June was planted and transplanted on the same dates.

According to table 1, eleven celery plants remained healthy and these were inoculated a second time during the spring. Ten of the plants developed symptoms of yellows as indicated in table 2. One plant grew a seed stalk and was rejected, since no study has been made of yellows in seed plants.

Transfer of Yellows from Experimentally and Naturally Infected Celery to Healthy Celery.—Non-infective six-spotted leafhoppers fed on some of the. celery experimentally infected with yellows (tables 1 and 2) and then on healthy celery, transmitted the disease to all healthy plants tested. Non-infective hoppers which were fed on healthy celery used as a check or control failed to produce the disease.

Non-infective six-spotted leafhoppers after feeding on thirteen naturally infected Golden Self-Blanching celery plants removed from the field in both early and late stages of the disease transmitted yellows to eight of thirteen healthy celery plants. Golden Heart and Yellow Plume were proved to be naturally infected with yellows.

Varieties of Celery Experimentally Infected with Yellows.—The following varieties of celery (*Apium graveolens dulce*) have been experimentally infected with the disease: Easy Blanching, Giant Paschal, Golden Self-Blanching (dwarf type and tall or new French type), and White Plume.

Celeriac or Turnip-Rooted Celery (Apium repaceum celeriac).— Celeriac or turnip-rooted celery was experimentally infected with yellows in the greenhouse.

Distribution of Disease in Celery Fields.—Celery affected with yellows was not uniformly distributed in a field, sometimes being more abundant along the margins. The diseased plants sometimes occur in groups scattered over several rows; frequently several adjacent plants in a row are diseased.

Fig. 7. Left, malformed leaf of plantain or ribgrass (*Plantago major*), show-ing yellowing. Right, dark green, healthy leaf.

Infection of Celery from Plantain Affected with Yellows (Plan-tago major).—Plantain or ribgrass (*Plantago major*) growing in the irrigation furrows between the celery beds at Terminus was proved to be naturally infected with yellows. Non-infective six-spotted leafhop-pers after feeding on plantain transmitted yellows to celery and asters. The inner leaves of this diseased weed were often malformed and chlo-rotic (fig 7) with elongated petioles, while the youngest leaves were

Fig. 8. Left, healthy spike of plaintain or ribgrass (*Plantago major*). Four spikes on right affected with yellows, three of them showing curling.

reduced in width, being narrow and elongated. The inner spikes were yellow, dwarfed, and sometimes curled (fig. 8). Nymphs and adults of the six-spotted leafhopper were commonly observed on plantain or rib-grass. Nymphs were hatched from eggs deposited in this weed under natural conditions and completed their life history on it in the green-house. In all probability, the six-spotted leafhoppers transmitted yellows from plantain or ribgrass to celery, since nymphs and adults were common on celery along the margin of the beds.

Absence of Yellows in Celery Beds.—A number of celery beds were examined in the San Joaquin delta and Salinas Valley but no yellows has been found up to the present time. Some celery growers plant celery after harvesting a crop of potatoes, but celery beds exam-ined as late as July 10, failed to reveal a single plant affected with yellows. As soon as celery plants make a thrifty growth after trans-planting, yellows appears. In all probability when the plants are grown close together in the celery beds, the symptoms fail to develop.

Early and Late Planting of Celery.—Celery growers state that early planted celery shows a higher percentage of yellows than late plantings in the San Joaquin delta. In the Santa Clara Valley, how-ever, one celery grower was supplying the local markets with celery during the winter, and repeated examinations of his field showed that 25 per cent of his crop was affected with yellows.

Mechanical Inoculation Tests.—All attempts to transmit yellows to celery by diseased juice inoculations have failed. In one experiment sixty-one healthy celery plants were inoculated with juice extracted from the roots, stems, petioles, or blades of ten naturally infected celery plants, using the flamed needle or capillary tube method of inoculation, but all without effect. In the next experiment forty-two healthy celery plants were inoculated with filtered juice extracted from the roots, stems, and petioles of eight celery plants experimen-tally infected with yellows. The juice was centrifuged for one hour and filtered through fine Berkefeld candles. A 5 per cent solution of hypophysis was added to some of the diseased juice which was then filtered through fine Berkefeld candles. The filtered juice in sterilized test tubes was capped with a mixture of equal parts of vaseline and paraffin and incubated for a period of from two to ten days before inoculation. It was then injected with a hypodermic syringe into the stem and root of healthy celery plants, but without effect.

ASTER YELLOWS

Smith[11] has described the aster yellows disease, especially as it affects the flower, and Kunkel[4] described the foliage symptoms. A detailed description is not necessary in this paper, but a brief account of the more important symptoms will be given.

Fig. 9. China aster (*Callistephus chinensis*) affected with yellows, showing upright petioles and dwarfed younger leaves. Non-infective six-spotted leafhoppers, after feeding on diseased celery plants, transmitted yellows to asters.

Symptoms on China Aster.—The first symptom to appear on the youngest leaves of a small plant is a clearing or transparency of the veinlets (pl. 1, figs. 1, 2) with a slight yellowing along them. Diseased leaves are frequently malformed (pl. 1, fig. 2) with somewhat longer, upright petioles (fig. 9). The youngest leaves are dwarfed with shortened petioles and the blades are sometimes reduced to but little more than the diameter of the petioles (fig. 10). Small plants when infected are severely stunted, but the amount of dwarfing of a plant varies with the size of the plant at the time of infection. Secondary shoots frequently grow in the axil of the leaves.

One of the most striking symptoms of aster yellows is the peculiar abnormal development of the flowers. Greenish yellow flowers frequently develop which are often dwarfed (fig. 11). A portion of a flower may show the natural color of the variety while the remaining part may be greenish yellow or white (fig. 12). The secondary shoots arising from the axil of the leaves usually bear no flowers or only small flower heads. Diseased flowers develop an enlargement of the ovaries (pl. 2) but produce little or no seeds.

Fig. 10. Terminal end of two China aster (*Callistephus chinensis*) plants affected with yellows, showing dwarfed youngest leaves with shortened petioles. The youngest leaves of the plant on the right have blades reduced to but little more than the diameter of the petioles.

Transfer of Celery Yellows to China Aster.—Non-infective six-spotted leafhopper after feeding on experimentally and naturally infected celery transmitted the disease to healthy China asters. Non-infective hoppers after feeding on asters experimentally infected with yellows transmitted the disease back to healthy asters and celery. When non-infective leafhoppers were transferred from healthy celery or asters to aster plants, yellows did not develop. This experiment proves that the virus of celery and aster yellows are identical.
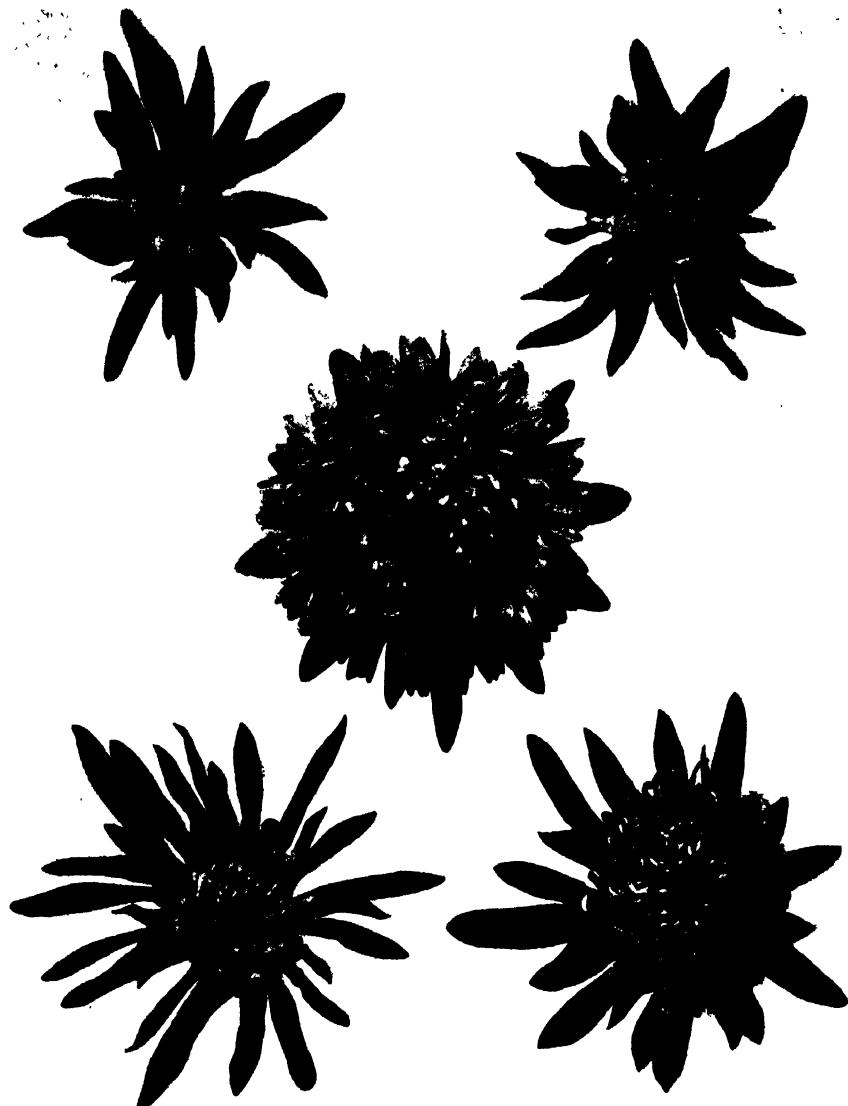
Fig. 11. Center: normal flower, from a China aster (*Callistephus chinensis*) plant to which non-infective six-spotted leafhoppers failed to transmit the disease from healthy celery. Grouped around it are four dwarfed aster flowers which were greenish yellow in color, from a plant infected with yellows by the six-spotted leafhopper, transferred from diseased field celery.
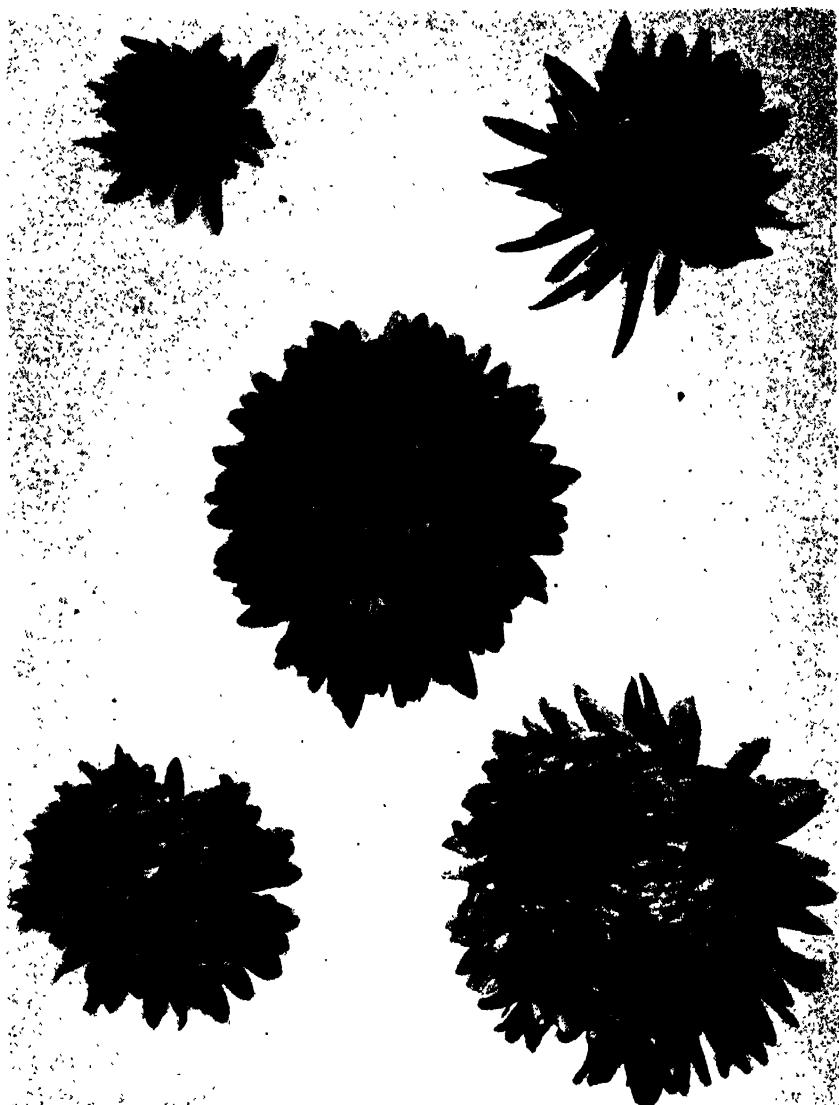
Fig. 12. Center: normal flower, from a China aster (*Callistephus chinensis*) plant to which non-infective six-spotted leafhoppers failed to transmit the disease from healthy celery. Grouped around it are four flowers showing abnormal portion which was greenish white or white, while the normal part retained the natural color of the variety; from aster plants inoculated with yellows by non-infective six-spotted leafhoppers after feeding on celery infected with the disease in the greenhouse.

Incubation Period.—The first symptom of aster yellows to develop, namely, the cleared or transparent veins, appeared in from 11 to 27 days in small plants, with an average period of 18.3 days in the greenhouse, as indicated in table 3.

TABLE 3

INCUBATION PERIOD OF YELLOWS DISEASE IN ASTERS IN THE GREENHOUSE

Aster plant No.	Dates *C. sexnotata* inoculated plants	Date transparent venation developed	Incubation period in plant *days*
1	Jan. 14	Feb. 5	22
2	Feb. 2	Feb. 19	17
3	Feb. 6	Feb. 19	13
4	Feb. 6	Feb. 19	13
5	Feb. 6	Feb. 26	20
6	Feb. 6	Feb. 26	20
7	Feb. 6	Feb. 26	20
8	Mar. 11	Mar. 30	19
9	Mar. 27	Apr. 23	27
10	Apr. 7	Apr. 18	11
11	Apr. 7	Apr. 27	20
Average			18.3

YELLOWS OF OTHER FLOWERING PLANTS

Zinnia.—A circular bed of zinnias (*Zinnia elegans*) showing 100 per cent yellows was found in the center of a lawn in front of the Spreckels Agricultural Experiment Station. The six-spotted leafhopper was abundant on the zinnias and on the grass. The plants were stunted, chlorotic, and with abnormal flowers (fig. 13). Noninfective leafhoppers after feeding on the diseased zinnias transmitted yellows to asters and celery. A shipment of zinnias was also received from San Gabriel, Los Angeles County, and these were also proved to be naturally infected with yellows. Zinnias grown from seeds were also experimentally infected with the disease.

African Marigold.—African marigold (*Tagetes erecta*) was demonstrated to be naturally infected with yellows in Berkeley. The plants were stunted and yellow and failed to blossom.

A list of flowering plants naturally and experimentally infected with yellows and curly top will appear in a future paper.

Fig. 13. Malformed flowers of *Zinnia elegans* affected with yellows.

LETTUCE YELLOWS (WHITE-HEART, RABBIT-EAR, OR RIO GRANDE DISEASE OF LETTUCE)

Kunkel[4] considers aster yellows identical with a serious malady of lettuce (*Lactuca sativa*) known as white-heart or rabbit-ear in New York and Rio Grande disease in Texas.

The variety of lettuce known as New York or Los Angeles was proved to be naturally infected with yellows in the Santa Clara and Salinas valleys. Non-infective six-spotted leafhoppers were fed for a few days on diseased lettuce removed from the field and were then transferred to healthy lettuce, celery, and asters, and all developed typical symptoms of yellows.

Lettuce yellows was rare in the Santa Clara Valley. An examination of the lettuce fields in the Salinas Valley was made on June 7–8, 1928, and lettuce affected with yellows was found here and there in a few rows adjacent to alfalfa and barley fields, but the disease was rare away from the margin of the lettuce fields. The six-spotted leafhopper was abundant in alfalfa and barley fields and was common in the lettuce fields. In the Watsonville district, lettuce yellows was somewhat more abundant than in the Salinas district, but the disease cannot be considered of economic importance at present.

Symptoms.—Lettuce affected with yellows before heading is readily detected at a short distance in the field by the yellow color of the outer leaves, the blanched appearance of the heart leaves, and a stunting of the plant. (pl. 3). The blanched inner or youngest leaves are dwarfed and the blades are often reduced to but little more than the petioles (fig. 14). In lettuce infected before heading, the heart leaves curl outward instead of inward and form no heads (pl. 4).

Lettuce affected after heading showed the dwarfed blanched heart leaves (pl. 5), which fail to form a solid head. Brown spots occur along the margin of the heart leaves (fig. 15), and sometimes the tips of the central dwarfed leaves are entirely brown (fig. 15).

Experimental Infection.—New York or Los Angeles lettuce experimentally inoculated with yellows by infective male *Cicadula sexnotata* in the greenhouse was transplanted in the field together with check or control plants on which non-infective leafhoppers had fed. The infected lettuce developed symptoms similar to those of naturally infected plants, while the check or controls remained healthy.
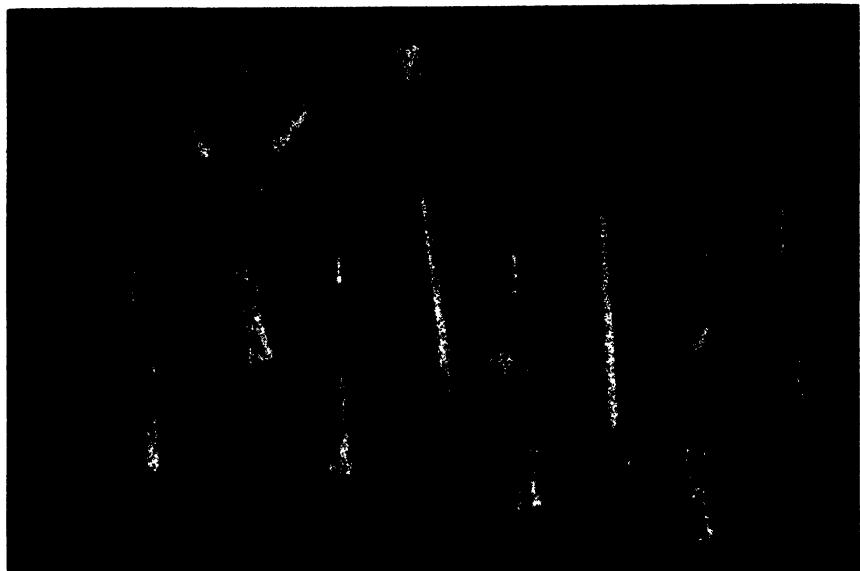
Fig. 14. Leaves from New York or Los Angeles lettuce (*Lactuca sativa*) plant inoculated with yellows by infective six-spotted leafhoppers, showing outward cupping of larger leaves, and dwarfing of smaller leaves with blades reduced to but little more than the petioles.

Fig. 15. Brown spots on heart leaves of New York or Los Angeles lettuce (*Lactuca sativa*) affected with yellows. Left, lower row: tips of two dwarfed leaves entirely brown.

Lettuce experimentally inoculated with yellows by infective six-spotted leafhoppers in the greenhouse assumed a spindling habit and developed many short upright flowering branches. The infected plants were chlorotic and showed the transparent veinlets on the younger leaves, although these cleared veinlets were often difficult to distinguish from normal venation of the leaves in the check or control plants. Non-infective six-spotted leafhoppers were fed for a few days on the experimentally infected lettuce and were then transferred to healthy lettuce, celery, and asters; all developed typical symptoms of yellows.

Varieties Experimentally Infected.—The following varieties of lettuce were experimentally infected with yellows: Big Boston, Chicken, Early Curled Simpson, Iceberg, New York or Los Angeles, Prizehead, Paris White Cos (Romaine, Cos, or Celery lettuce), and Wonderful.

SIX-SPOTTED LEAFHOPPER, *CICADULA SEXNOTATA* (FALL.)

Characteristics.—The adult insects (pl. 6, fig. 5) of the spring and summer broods are greenish yellow. A few specimens taken on the foothills of the Santa Clara Valley on February 28, 1928, possessed a faded light brown color pattern resembling in color somewhat the dark overwintering beet leafhoppers (pl. 6, figs. 1–4) near the end of their natural life. The adults are easily recognized by the six black spots (pl. 6, fig. 5) on the vertex of the head, and the front has a double series of black arcs.

The nymphs after hatching develop a dusky color, and in later instars, the general color varies from yellow to light brown, or light greenish-gray.

Distribution.—According to Osborn[7] the widely distributed six-spotted leafhopper was described in Europe more than a century ago. Uzel[10] reported this species as injurious to sugar beets in Bohemia. Ellinger[2] records it as injurious to wheat, oats, and barley in Sweden. According to Junger,[3] it is well known throughout Germany as the cause of severe injury to grasses, cereals and certain legumes. Kunkel[4] states that it occurs in Japan and probably throughout the Orient.

Osborne[9] found a specimen of *Cicadula sexnotata* in the Harris collection in the Boston Society of Natural History probably collected

between 1840 and 1850, but there was no published record of its occurrence in the country prior to 1884, a fact that very naturally suggests that it might be an introduced species. It is now widely distributed in North America from Alaska to Florida and from Maine to California.

The six-spotted leafhopper is generally distributed in California. Specimens were collected as far south as Calexico in the Imperial Valley as follows: April 2, 1918, on pepper grass (*Lepidium medium*) *Malva* sp. and *Rumex* sp.; in Heber, April 3, 1918, on wheelscale (*Atriplex elegans*). During the past ten years it has been taken on pasture grasses on the plains and foothills of the San Joaquin Valley, also on the foothills bounding the Salinas and Santa Clara valleys.

Flights.—Field observations made in the San Joaquin and Santa Clara valleys during the winter and spring of 1928, indicate that the six-spotted leafhopper flies into the cultivated areas after the pasture vegetation becomes dry on the plains and foothills. Spring brood adults were common during March in fields of barley and oats adjacent to the foothills in the San Joaquin Valley, but during the middle of April, after the grain begins to ripen, the adults fly to other food plants.

Food Plants—The six-spotted leafhopper has a wide range of food plants in the United States and is a pest to forage, cereal, garden crops, and flowering plants. Osborn[8] found it abundant in meadow and marsh grasses and pastures, and common on timothy in Maine. It migrates to grain fields and is a serious pest to oats. After the oats ripen it spreads to adjacent potato and corn fields. Kunkel found that *Cicadula sexnotata* will live and reproduce on "aster, lettuce, sow thistle, great ragweed (*Ambrosia trifida* L.) daisy fleabane (*Erigeron annuus* (L.) Pers.) and other Erigerons, English plantain (*Plantago lanceolata* L.) dandelion (*Taraxacun officinale* Weber), wheat, oats, rye, barley calendula, *Ammobium alatum, Matricaria alba, Centaurea imperialis, Gaillardia grandiflora,* Moon Penny daisy (*Chrysanthemum leucanthemum*), and the African daisy (*Dimorphotheca aurantiaca*)."[4]

Life History on Celery.—The six-spotted leafhopper completed its life cycle on young and half-grown Golden Self-Blanching celery plants, but older plants are unfavorable for the multiplication of this insect.

Mortality on Celery.—A high mortality of the adults occurs on large celery plants. In one experiment 120 adults were distributed on sixteen large celery plants enclosed in cages on December 4, and

only 25 adults were alive on February 4. As there was a possibility that the leafhoppers were at the end of their natural life, another experiment was performed.

Two small celery plants about transplanting size and two large celery plants were enclosed in four cages, each containing 25 male or female leafhoppers. The mortality at intervals of five or ten days is shown in table 4.

TABLE 4

COMPARISON OF MORTALITY OF SIX-SPOTTED LEAFHOPPERS ON LARGE AND SMALL CELERY PLANTS

Dates	Males		Females	
	On large plant	On small plant	On large plant	On small plant
June 2 . . .	25	25	25	25
June 12	2	17	10	18
June 22	1	15	5	11
June 27	1	13	1	9
July 2 . .	0	12	1	8
July 7 . . .	0	9	0	6

Life History on Lettuce.—Nymphs which hatched from eggs deposited by the six-spotted leafhopper in the following varieties of lettuce completed their life cycle on these host plants in the greenhouse: Big Boston, Chicken, Early Curled Simpson, Iceberg, New York or Los Angeles, Prizehead, Paris White Cos (Romaine, Cos, or Celery lettuce), and Wonderful.

Overwintering Stage.—Experiments conducted by Kunkel[4] give indirect proof that the six-spotted leafhopper passes the winter in the egg stage in New York. This leafhopper winters over in the adult stage in California, deposits its eggs, and is at the end of its natural life in March. Nymphs in the last instar were taken on the foothills of the Santa Clara Valley on February 28, 1928. A few females with a faded light brown color pattern were also taken, but these died in a few days on asters. Adults were also taken during December, 1928, and January, 1929, wintering on the foothills and in the cultivated areas of the Salinas Valley.

Transmission Experiments with Sugar-Beet Curly Top.—The six-spotted leafhopper is not able to transmit curly top. Non-infective *Cicadula sexnotata* after feeding on curly-top beets failed to communicate this disease to celery plants, asters, and beets. Non-infective six-spotted leafhoppers did not transmit curly top from two celery plants experimentally infected with the disease to healthy beets.

Life History and Longevity on Sugar Beets.—The six-spotted leaf-hopper was not able to complete its life history on sugar beets. The longevity of the last living male and female leafhopper was determined with different lots of adults feeding on small and large beets as follows:

Sugar beets	Longevity of males *days*	Longevity of females *days*
2–4 leaves	2 11	2–4
8–10 leaves	7	60
12–16 leaves	22	62
4 months old	14	18

It is evident that the adult life of the males was shorter than the females on all beets except those with 2–4 leaves.

EXPERIMENTS WITH BEET LEAFHOPPER, *EUTETTIX TENELLUS* (BAKER)

Investigations conducted in the celery fields in the San Joaquin delta regions and in the vegetable gardens of the Spreckels ranches in the Salinas Valley during the 1925 outbreak of the beet leafhopper, *Eutettix tenellus* (Baker), demonstrated that this insect was occasionally taken on celery. In years between outbreaks of the pest, this leafhopper was rarely found on celery. During the spring of 1927 the beet leafhopper migrated over the Coast Range from the San Joaquin into the Salinas and Santa Clara valleys, and an occasional adult, but rarely a nymph, dropped to the ground when celery was shaken during the summer.

A large number of celery plants were removed from the field during 1925–27, but, up to the present time, celery has not been demonstrated to be naturally infected with curly top.

Carsner[1] states that celery (*Apium graveolens*) is non-susceptible to curly top.

Golden Self-Blanching celery was inoculated with curly top by ten infective beet leafhoppers in the greenhouse but only two of eighteen plants were experimentally infected with the disease. Different lots of non-infective beet leafhoppers after feeding on the two plants infected with curly top were transferred to healthy beets and

typical symptoms of the disease developed. In one celery plant infected with curly top during April, the virus remained active during May and June, while from the second celery plant the disease was repeatedly transmitted to beet seedlings for a period of six months and at the present writing the virus is still active. Celery experimentally infected with curly top showed a shortening of the petioles of the central leaves but the petioles failed to twist and intertwine as in celery yellows. The infected plants showed the transparent veinlets on the youngest leaves, but these cleared veinlets were difficult to distinguish from normal venation in the check or control plants.

Giant Paschal and White Plume celery were also inoculated with curly top by ten infective beet leafhoppers but two of six plants of the former variety, and one of six plants of the latter variety were experimentally infected with the disease.

Nymphs which hatched from eggs deposited by the beet leafhopper in Giant Paschal, Golden Self-Blanching, and White Plume celery completed their life cycle in the greenhouse.

The beet leafhopper is not able to transmit yellows and the beet is immune from this disease. Non-infective beet leafhoppers after feeding on celery and asters affected with yellows failed to transmit this disease to healthy celery plants, asters, and beets. Healthy beets were repeatedly inoculated with yellows by different lots of infective six-spotted leafhoppers for a period of three weeks, but non-infective *Cicadula sexnotata* or *Eutettix tenellus* after feeding on the inoculated beets failed to transmit this disease to healthy celery plants, asters, and beets.

Asters were repeatedly inoculated with curly top by different lots of infective beet leafhoppers but asters were found to be immune from this disease.

Nymphs which hatched from eggs deposited by the beet leafhopper in asters failed to complete their life history in the greenhouse. The males lived from 3 to 11 days and the last female died at the end of 17 days on asters.

The following varieties of lettuce were demonstrated to be immune to curly top: Big Boston, Chicken, Early Curled Simpson, Iceberg, New York or Los Angeles, Prizehead, Paris White Cos (Romaine, Cos, or Celery lettuce), and Wonderful. The beet leafhopper failed to complete its life cycle on the above varieties of lettuce.

EXPERIMENTS WITH *AGALLIA CALIFORNICUM* (BAKER),
A. CINEREA (O. & B.), AND *EMPOASCA*
FLAVESCENS (FAB.)

An examination of the insect population on celery was made during 1925 and in later years. Several other species of leafhoppers were often taken on celery. Nymphs and adults of *Agallia californicum* (pl. 6, fig. 6), *A. cinerea,* and *Empoasca flavescens* (pl. 6, fig. 7), were taken on celery in the field. The three species of leafhoppers completed their life cycle on celery in the greenhouse and many generations were reared.

Adults of each species bred on celery affected with yellows were transferred to healthy celery and aster plants, but in no case did they transmit the disease.

SUMMARY

The data presented in this paper prove that the six-spotted leaf-hopper *Cicadula sexnotata* (Fall.), transmits yellows disease to celery. Non-infective six-spotted leafhoppers reared on barley failed to produce the disease in healthy celery. The disease was transferred by non-infective leafhoppers feeding on celery experimentally infected in the greenhouse back to healthy celery. Non-infective leafhoppers after feeding on naturally infected celery transmitted yellows to healthy celery plants.

It was demonstrated that yellows of celery is identical with aster yellows. Non-infective six-spotted leafhoppers after feeding on experimentally and naturally infected celery transmitted yellows to asters. Non-infective hoppers after feeding on asters experimentally infected with yellows transmitted the disease back to healthy celery and asters.

It was also shown that yellows of celery and asters is identical with lettuce yellows, which is also known as white-heart, rabbit-ear, or Rio Grande disease.

A number of flowering plants of the Compositae, such as China asters, zinnias, and African marigold, were proven to be naturally infected with yellows. Plantain or ribgrass (*Plantago major.*) was also demonstrated to be naturally infected with yellows.

Celery and aster yellows first made its appearance in California during 1925, and in four years has spread from Sonoma to Los Angeles counties.

ACKNOWLEDGMENT

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PLATES 1-6

PLATE 1

China Aster (*Callistephus chinensis*)

Fig. 1. Clearing or transparency of the veins on left half of leaf affected with aster yellows.

Fig. 2. Malformed and dwarfed leaves of asters affected with yellows, showing the clearing and transparency of the veins on a portion of the blade.
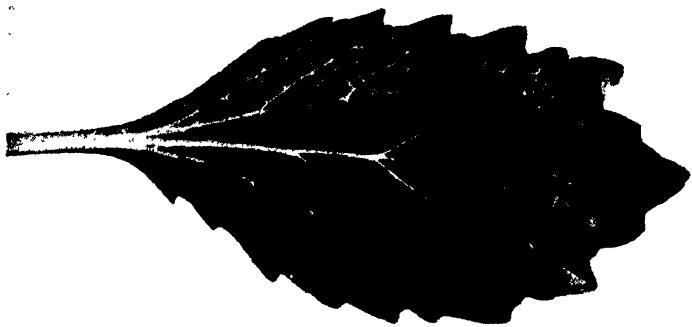
Fig. 1

Fig. 2

China Aster (*Callistephus chinensis*)

Longitudinal sections of aster flowers: center, from a healthy plant; others, from diseased plants showing enlarged ovaries.

New York or Los Angeles Lettuce (*Lactuca sativa*)

Upper, healthy lettuce head. Two lower lettuce plants were naturally infected with yellows, also known as white-heart, rabbit-ear, or Rio Grande disease. The two stunted plants were transplanted on the same date as the healthy one.

New York or Los Angeles Lettuce (*Lactuca sativa*)

Lettuce plant naturally infected with yellows, showing dwarfed youngest leaves, and outwardly curled older leaves. This is the same plant as that shown in the lower left-hand corner of plate 3, enlarged to show the symptoms.
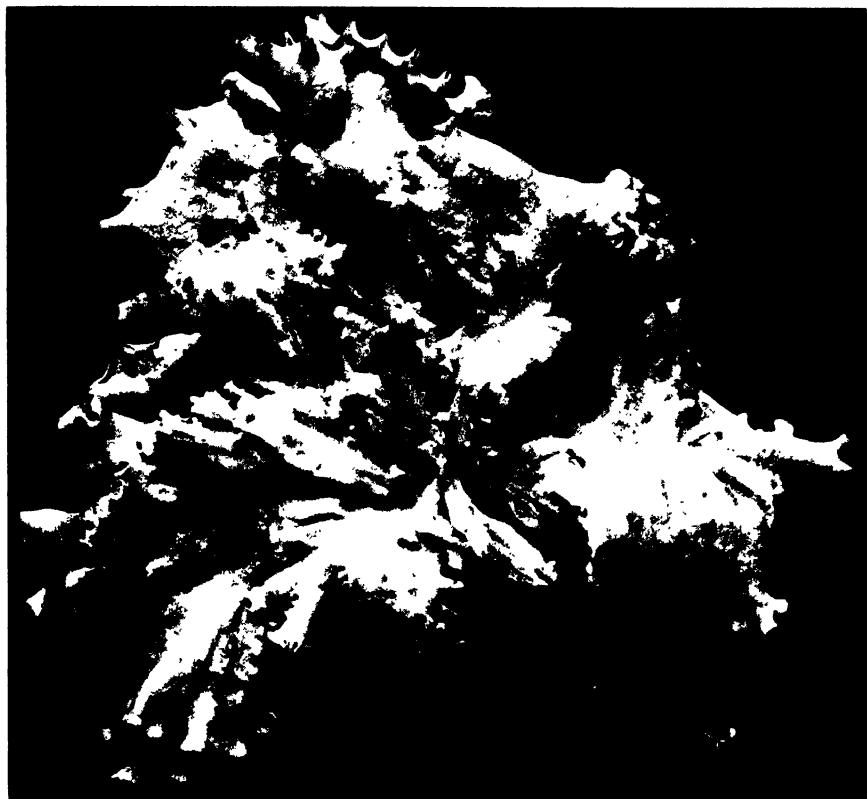
PLATE 5

New York or Los Angeles Lettuce (*Lactuca sativa*)

Longitudinal sections of lettuce heads: upper, healthy; lower, naturally infected with yellows, showing dwarfed, outward-curled, central leaves.

Fig. 1   Fig. 2   Fig. 3   Fig. 4

Fig. 5   Fig. 6   Fig. 7

A MACROSCOPICAL ANALYSIS OF THE FLEECES OF FOUR ROMNEY RAMS

J. F. WILSON[1]

Improvement in character of fleece in any flock is largely dependent upon the judicious selection of rams. It is necessary to assume that the fleece character possessed by the sire will in some measure be transmitted to the offspring. In judging rams for quantity and quality[2] of fleece, it is necessary, therefore, to make direct comparisons, and to assume that the offspring of a ram possessing an excellent fleece will have better fleeces than the offspring of another ram possessing a fleece less desirable.

Judging the excellence of a fleece by simple optical examination has been in the past the only method employed by the practical breeder. This method of judging is satisfactory in so far as it concerns the animal and its body characters, but when used for judging fleeces, it is subject to certain very definite limitations and often to serious error. It is quite impossible to judge optically, with any satisfactory degree of accuracy, the variation in the diameter of the fiber; yet uniformity of diameter is one of the characters most closely correlated with the spinning properties of wool. Similarly, judging the general fineness of wool with the eye may be subject to large error. The breeder is apt to correlate too closely the fineness of fiber

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[2] "Quality," as used here, refers to all of the characteristics of the fleece other than weight and length. It has no reference to the orthodox definition— diameter of fiber.

with the number of crimps per inch, and while crimp is generally associated with fineness, some exceptionally fine wools nevertheless show very little crimp. This fact is exemplified in a fleece now in the possession of the writer. The fleece was produced by a ram of the Australasian Merino × Rambouillet cross. Its lack of well defined crimp and its general bold style indicate on superficial examination about a 64's to 70's fineness. Actual measurement of 300 fibers of shoulder wool with the micrometer caliper, however, showed them to have a mean diameter of slightly less than .0003 inch, or finer than the finest Silesian Merino recorded in Bowman's "The Structure of the Wool Fiber."[1] Most Rambouillet fleeces have a mean diameter of about .0006 inch.

The tests herein described were carried out in the hope that they might lead to a method of judging the fleeces of breeding sheep more accurately than is possible by simple examination. It is realized that the technique involved in this entire study is too laborious to warrant its practical application except, perhaps, to high class stud animals in the best registered flocks. The data indicate, however, that this or some similar method of procedure might prove valuable in the selection of rams of some breeds. A study of the mean diameter, and probable error of the mean, together with a study of the percentage of medullated fibers, in the shoulder and thigh wool of long-wool rams would not involve too much time and would yield valuable information.

The fleeces used for these analyses were furnished by Dr. E. E. Brownell, a Romney breeder of Woodland, California. Three of the four rams which produced the fleeces were imported directly from New Zealand, while the fourth, Brownell 39, was imported in dam from the same country (figs. 1 and 2).

DIAMETER OF FIBER

Samples were taken from ten different places on each ram as follows (fig. 3):

1. Shoulder: about three inches to the rear of the point of the shoulder.
2. Side: at the intersection of two imaginary lines drawn between the withers and flank and between the elbow and hip.
3. Thigh: about two inches above and to the side of the hock.
4. Neck: midway between the brisket and the angle of the jaw.
5. Ear: immediately back of the ear.

6. Cheek: as nearly in the center of the cheek as possible.

7. Back *1*: between the shoulder blades.

8. Back *2*: between the hips.

9. Belly: about two inches in front of the sheath.

10. Scrotum: about the center of the front side of the scrotum.

The samples were washed in benzene to remove natural impurities and were measured at the midsection with a Brown and Sharpe micrometer, reading directly to 1/10,000 of an inch (fig. 4).

The data in table 1 show conclusively that the shoulder wool of these Romney rams was not the finest found in the fleece. This fact is at variance with the statements of "Shepherd Boy,"[2] Hawkesworth,[3] Matthews,[4] and Horlacher[5] that the finest wool in the fleece is found on the shoulder. On three of the four rams, the finest wool was found on the ear or the cheek, while the fourth ram produced the finest wool on the scrotum. Mathematical calculation, by the "Student"[6] method, shows that in these rams, the odds are 47 to 1 that the wool from the ear is finer than that from the shoulder.

It has been customary to consider that the coarsest wool in the fleece invariably comes from the thigh, yet these data indicate that this may not always be true.

The probable errors indicate in a general way the uniformity of diameter of fiber. The data show that in three of the fleeces, the thigh wool was the least uniform, while in the fourth fleece, from the Short ram, the belly, back *2*, and the side were all relatively non-uniform. The ear and cheek samples showed in general the greatest uniformity.

TABLE 1

DIAMETERS OF FIBERS OF ROMNEY RAMS IN TEN-THOUSANDTHS CF AN INCH

Name of ram	Mean diameter of 100 fibers from:										Mean of all samples
	Shoulder	Side	Thigh	Neck	Ear	Cheek	Back *1*	Back *2*	Belly	Scrotum	
Brownell 39 ......{	9.09 ±1.26	10.11 ±1.12	11.12 ±1.51	10.56 ±1.06	8.14 ±.86	8.42 ±.99	9.97 ±1.26	11.67 ±1.07	10.95 ±1.32	10.07 ±1.42	10.01
Short 315 ....{	10.08 ±1.11	11.00 ±1.76	12.80 ±1.58	9.42 ±1.13	7.90 ±.69	8.18 ±.70	10.47 ±1.21	11.99 ±1.77	13.55 ±1.77	11.81 ±1.17	10.72
Matthews 39......{	10.90 ±1.39	12.40 ±1.26	14.80 ±1.73	11.16 ±1.02	10.62 ±1.30	10.95 ±1.17	11.41 ±1.25	13.77 ±1.62	13.68 ±1.63	10.10 ±1.03	11.99
Goulter 106.........{	10.19 ±1.35	10.38 ±1.32	13.67 ±1.75	11.42 ±1.30	9.25 ±1.01	7.94 ±1.18	10.24 ±1.53	11.07 ±1.45	12.78 ±1.46	10.78 ±1.13	10.76

Fig. 1. Rams whose fleeces were used in this experiment.
Upper, Brownell 39; lower, Short 315.

Fig. 2.   Rams whose fleeces were used in this experiment.
Upper, Matthews 139; lower, Goulter 108.

Fig. 3. Showing where the samples were taken.

Fig. 4. Type of micrometer used for measuring the diameters of the
wool fibers.

PERCENTAGE OF MEDULLATED FIBERS

The medullated or tubular wool fiber occurs in nearly all breeds of sheep, but is found most frequently among the long-wool breeds. It is considered a serious defect in the fleece and is thought to be responsible for harshness of the wool, poor dyeing properties, and lack of elasticity.

The method of detecting the medullated fiber macroscopically has been previously described by the writer.[7]

Medullation of the wool fiber may be complete from the proximal end to the distal end; it may be intermittent to any degree, or entirely absent.

Table 2 indicates that the medullated fiber occurred most frequently in the rear portions of the fleece. The side, thigh, back 2, and belly contained by far the largest proportions of medullated and partly medullated fibers, while the shoulder, neck, ear, cheek, and back 1 were comparatively free from the presumed defect. Comparison of table 1 with table 2 shows that the coarser parts of the fleece contained the highest proportions of medullated fibers.

TABLE 2

NUMBERS OF MEDULLATED AND NON-MEDULLATED FIBERS IN VARIOUS PARTS OF FLEECES OF ROMNEY RAMS

Name of ram	100 fibers from:										
	Shoulder	Side	Thigh	Neck	Ear	Cheek	Back 1	Back 2	Belly	Scrotum	*Totals*
Brownell 39											
Non-medullated........	95	59	38	88	100	100	100	52	46	70	*748*
Partly medullated....	5	39	29	12	0	0	0	48	34	27	*194*
Medullated.......	0	2	33	0	0	0	0	0	20	3	*58*
Short 315											
Non-medullated........	63	52	42	74	99	97	83	50	42	92	*694*
Partly medullated...	31	36	47	24	1	3	17	29	53	8	*249*
Medullated..............	6	12	11	2	0	0	0	21	5	0	*57*
Matthews 139											
Non-medullated........	92	78	41	96	100	97	78	53	61	96	*792*
Partly medullated....	8	22	55	4	0	3	22	46	37	4	*201*
Medullated..............	0	0	4	0	0	0	0	1	2	0	*7*
Goulter 108											
Non-medullated.......	85	46	48	79	96	72	84	81	44	43	*678*
Partly medullated....	14	50	40	20	4	21	16	19	49	55	*288*
Medullated..............	1	4	12	1	0	7	0	0	7	2	*34*
Totals											
Non-medullated........	335	235	169	337	395	366	345	236	193	301	*4000*
Partly medullated....	58	147	171	60	5	27	55	142	173	94	
Medullated................	7	18	60	3	0	7	0	22	34	5	

If the medullated fiber is to be considered a serious defect, Brownell 39 and Matthews 139 have fleeces superior to the two other rams. Judging by simple optical examination, however, Short 315 would be rated as high as the Matthews ram, although the fleece of Brownell 39 was outstanding. The fleece of Goulter 108 would be considered easily the poorest fleece of the four.

RATIO OF STAPLE LENGTH TO FIBER LENGTH

The purpose of this phase of the analysis was to attempt to show arithmetically the degree of crimp in the wool. The method usually employed of ascertaining the number of crimps per inch does not indicate the degree or definiteness of crimp. Thus two fibers of fine wool may each have twelve crimps to the inch. But one fiber may have the crimp exceptionally well defined, while in the other the crimps may be so slight as to be almost negligible. · Obviously the fiber with the well defined crimp is more desirable, other things being equal, since it would be the longer when straightened out and would, therefore, possess superior spinning properties. The ratio of staple length to fiber length gives no indication of the number of crimps to the inch, but determines roughly whether or not the crimp is well defined.

For this test the staple as it came from the fleece was placed on a photograph trimming board and cut off at both the proximal and distal ends in a manner which left a section of the staple exactly two inches long. These two-inch samples, taken from the midsection of the staple, were used in the study of the ratio of staple length to fiber length. The actual length of the fiber is far greater than the apparent length, on account of the crimp. One hundred fibers were drawn from each of the two-inch bundles, and the actual length of each fiber was measured. The results are presented in table 3.

TABLE 3

Ratio of Staple Length to Fiber Length in Fleeces of Romney Rams

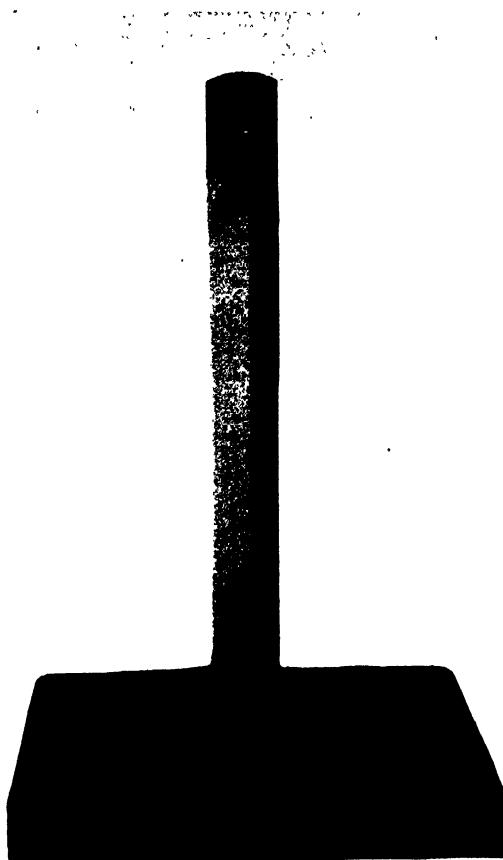| Name of ram	100 fibers from:									Average of all samples
	Shoulder	Side	Thigh	Neck	Ear	Cheek	Back 1	Back 2	Belly	
Brownell 39	1:1.20	1:1.35	1:1.70	1:1.15	1:1.45	1:1.40	1:1.30	1:1.50	1:1.35	1:1.38
Short 315.........	1:1.30	1:1.35	1:1.90	1:1.45	1:1.25	1:1.40	1:1.40	1:1.55	1:1.35	1:1.44
Matthews 139...........	1:1.45	1:1.30	1:1.95	1:1.20	1:1.25	1:1.25	1:1.40	1:1.45	1:1.40	1:1.40
Goulter 108..............	1:1.30	1:1.35	1:1.80	1:1.45	1:1.25	1:1.38	1:1.35	1:1.40	1:1.60	1:1.43

Fig. 5. Device for measuring the actual length of wool fibers.

The apparatus used to measure the length of the fibers was devised by the writer (fig. 5).

The thickness of the jaws of the upper clamp is $\frac{1}{16}$ of an inch. The scale is placed in such a position that the "0" corresponds to the upper edges of the jaws of the clamp. The end of the fiber is pulled through the jaws until the tip is flush with the upper edges of the jaws, after which the clamp, containing the fiber, is placed on the brass peg. A small specially designed 'safety pin' clamp, made of piano wire, is then attached to the lower end of the fiber in such a way that the lower fiber tip is flush with the lower side of the jaws of the pin. This 'safety pin' clamp weighs 0.8 of a gram and is just heavy enough to remove the crimp from the Romney fiber without

stretching it perceptibly. By its use the error attendant upon stretching the fibers straight with the hands is eliminated, since the apparatus gives a uniform tension to each fiber.

The data presented above are in the main inconclusive and inconsistent. They show, however, that the wool from the thigh was more boldly crimped than that from any other portion of the fleece measured. This is rather surprising in view of the fact that the thigh samples in three of the four rams appeared to have less pronounced crimp than was found among most of the other samples from the same sheep. Generally speaking, the finer portions of the fleeces showed a larger ratio of staple length to fiber length, though there were some exceptions.

SUMMARY

It should be borne in mind that this paper deals only with the fleeces of four individuals of one breed. Before any satisfactory method of macroscopical analysis of fleeces can be evolved, it will be necessary to apply tests to fleeces from several breeds representing a wide range of wool types.

To complete the macroscopical analysis would require much work other than that herein described. Most particularly the clean or scoured weights of the fleeces, representing exactly twelve months' growth, should be obtained. A study of the density of the fleeces, by calculating the number of fibers to a square inch of skin surface on different parts of the body, would be valuable. As yet it has not been convenient to do this in connection with the present study. The results presented, however, indicate that breeders of stud sheep of some breeds might well adopt a method of studying fleeces other than by simple examination. The micrometer should not supplant the breeder's individual judgment, but should aid him in formulating an opinion of the merits of the fleece. The test for medullated fibers is so simple that it can, with a little practice, be performed by any intelligent breeder, and the technique involves only a few cents for equipment. If these hair-like fibers are to be eliminated from the coarse-wool breeds of sheep, the elimination must take place through proper selection of breeding sires. The shoulder and thigh wool from such animals might be tested for percentage of medullated fibers. It is doubtful if the ratio of staple length to fiber length is of value in a study of fleeces from the long-wool breeds, although it might yield interesting information if applied to the fine-wools.

Selection of sheep by optical examination has in the last 100 years resulted in an enormous increase in fleece weights. Indications are that the limit of such weights is still a long way from realization, although the law of diminishing returns is probably now retarding progress. It is probable also that during the same length of time considerable progress has been made in improving the character of the fleece. Here, however, we have no method of measuring achievement. The solution of problems of wool production most intimately associated with the quality of the finished cloth will probably demand a method of judging wool other than by the simple examination employed in the past.

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ADDITIONAL HOST PLANTS OF CURLY TOP

HENRY H. P. SEVERIN*

INTRODUCTION

In a recent paper,[9] the host plants of curly top in the families Chenopodiaceae, Leguminosae, and Cucurbitacea were given. Forty varieties of economic plants were reported to be naturally infected with curly top and 120 varieties were experimentally infected with the disease. Eight different species of weeds were demonstrated to be naturally infected with curly top, and nineteen weeds and shrubs to be experimentally infected with the disease.

During 1925 several varieties of peppers failed owing to curly top in the interior regions of California.[10] McKay[5] reported as high as 90 per cent of the peppers affected with curly top at The Dalles, Oregon, during 1926. Crawford[3] found a large percentage of Chili peppers affected with curly top in New Mexico during 1927.

According to Crawford† the experimental planting of tobacco (*Nicotiana rustica*) at the State College, New Mexico, was entirely destroyed by curly top. Tobacco was infected with the disease near Albuquerque. E. G. Beinharts also reported that tobacco was infected with curly top in Arizona.

During the 1925 outbreak of the beet leafhopper, horse-radish was demonstrated to be naturally infected with curly top in the Sacramento Valley.[6] According to McKay,[5] horse-radish was seriously infected with curly top in Oregon during 1926, and in some fields as high as 95 per cent of the crop was diseased.

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† Letter to author dated October 20, 1927.

In this paper, additional host plants of curly top in the families Solanaceae, Cruciferae, Umbelliferae, Malvaceae, Linaceae, Boraginaceae, and Valerianaceae are listed. Field investigations to determine the economic plants naturally infected with curly top were started during the 1925 outbreak of the beet leafhopper and continued over a period of four seasons. Investigations to ascertain the weeds which were naturally and experimentally infected with this disease was begun in 1918 and has been continued for a period of eleven years.

SOLANACEAE, NIGHTSHADE FAMILY

Potato (Solanum tuberosum).—Volunteer potato plants growing in a vegetable garden on the Spreckels ranch near King City were proved to be naturally infected with curly top during the 1925 outbreak of the beet leafhopper. Non-infective leafhoppers after feeding on 18 potato plants removed from the field transmitted curly top to beets from 14 of the plants. A diseased potato plant growing on the University Farm at Davis and determined as curly dwarf by J. T. Rosa was also demonstrated to be naturally infected with curly top.

A shipment of potato tubers grown on the University Farm was received on November 11, 1925, from J. T. Rosa, who wrote: "I am sending tubers of Irish potatoes that were exposed to leafhoppers and which became yellow and sickly, in my fall crop here. Will you plant them to test tuber transmission of curly top in potato?" The tubers were kept over winter, but curly top was not transmitted to beets during the following spring from any of the 12 potato plants grown. No tests had been made, however, during the autumn to determine whether the potato plants were naturally infected with the disease. Future experiments will show whether tuber transmission of curly top occurs in potatoes. It is known that the disease is carried over in stechlings and mother beets and seriously reduces seed production.

Twelve small potato plants growing on the University Farm at Davis were infected with curly top on May 17, using two leaf-cages each containing 10 nymphs to a plant. Each plant was again infected with the disease on May 30 and June 6, using a total of 60 nymphs. Non-infective beet leafhoppers after feeding on several tips removed from each plant were transferred to 12 sugar beets but only 3 developed curly top.

The following varieties of potatoes were experimentally infected with curly top: American Wonder, British Queen, Idaho Gems, Idaho Rurals, Red Prizetaker or Improved Early Rose, White Rose, White Rose Low-Top, and Wisconsin Pride.

The potato plants naturally infected with curly top were stunted, with yellowish, inward-rolled leaflets. Some of the potatoes infected with curly top on the University Farm showed an inward roll of the leaves, but after the potatoes were irrigated the plants appeared normal. Toward the end of June the leaves on the lower portion of a few of the infected potatoes turned orange yellow in color, but many plants not infected with the disease showed a similar discoloration of the foliage.

Fig. 1. White Rose Potato (*Solanum tuberosum*) experimentally infected with curly top, showing terminal shoots with inward-rolled leaflets and bent petioles.

In the greenhouse the eight varieties of potatoes experimentally infected with curly top also showed an inward roll of the leaflets and often a bending of the petioles (fig. 1). Potatoes in an advanced stage of the disease frequently developed dwarfed shoots in the axil of the leaves (fig. 2), near the tip of the plants. Later the plants turned yellow and died.

Tomato (Lycopersicon esculentum).—The proof that the beet leaf-hopper transmits curly top to tomatoes has appeared in a previous paper.[7]

Peppers (Capsicum frutescens).—During 1925, investigation of a field of Pimiento peppers grown near Freeport in the Sacramento Valley showed that 78 per cent of the crop was stunted, with thick, leathery inward-curled leaves. Curly-top beets had been plowed under

in the vicinity of this pepper field, forcing the beet leafhoppers to seek other food plants. Peppers similarly affected were also found in a field a few miles west of the city of Sacramento. Four varieties of peppers grown on the Spreckels ranch near King City were dwarfed; many of the plants were only 8 to 12 inches tall and often dry, while

Fig. 2. White Rose Low-Top potato (*Solanum tuberosum*) in an advanced stage of curly top, showing numerous dwarfed shoots and drying of some of the leaves.

others were about one-half of their normal size. Shipments of stunted Pimiento peppers grown by the California Packing Corporation at Armona, Kings County, in the San Joaquin Valley were sent to the University of California. Fruit was usually absent on the smallest plants but on the somewhat larger ones, small malformed fruit was

present (figs. 3, 4). Non-infective beet leafhoppers, however, did not transmit curly top from all of the dwarfed varieties of pepper plants removed from the field to sugar beets.

Fig. 3. Paprika pepper (*Capsicum frutescens*): stunted plant naturally infected with curly top, showing curled, dwarfed leaves on the terminal shoots, and malformed fruit.

The following varieties of pepper were proved to be naturally infected with curly top: Anaheim Chili, Paprika, Pimiento, and Mexican Chili.

When the four varieties of pepper naturally infected with curly top in the Salinas Valley were shaken, an occasional adult but rarely a nymph hopped from the plants.

Non-infective beet leafhoppers when allowed to feed on the fruit of naturally infected Anaheim Chili and Pimiento peppers with the stems removed transmitted curly top to sugar beets.

Fig. 4. Paprika pepper (*Capsicum frutescens*): fruit from healthy plant, and five dwarfed, malformed fruits from plant naturally infected with curly top.

The following varieties of peppers were experimentally infected with curly top: Anaheim Chili, California Wonder, Chinese Giant, Large Bell or Bull Nose, Long Red Cayenne, Mexican Chili, Pimiento, Red Chili, Royal King, Ruby King, Sweet Mountain, Sweet Upright, and Tobasco. The following varieties of pepper-tomato were also experimentally infected with the disease: Ignacio, Novata, Petaluma, San Geronimo, Sonoma, Topepo, and Tulare.

As a general rule, an inward curl of the youngest leaves and an outward cupping of the somewhat older leaves occurred in the first thirteen varieties of peppers listed. The veinlets became transparent on the youngest leaves. Transparent venation, however, has never been observed in naturally infected peppers, but no observations have been made on young plants. Minute swellings developed on the network of cleared veinlets resembling somewhat the warty protuberances on the leaves of sugar beets in an advanced stage of curly top.

In the seven varieties of infected pepper-tomatoes, some of the leaflets showed an inward curl, and sometimes the petioles of two adjacent leaflets were bent so that the upper surfaces of the leaflets were in contact or the youngest leaves were very much twisted (pl. 1). White swellings sometimes appeared on the lateral veins of the youngest leaves. A yellowing developed between the lateral veins, while the veins remained green. In the later stages of the disease the plants were decidedly yellow.

The longevity of the last living male and female beet leafhopper on the different varieties of peppers was as follows:

Variety of pepper	Longevity of males *days*	Longevity of females *days*
Anaheim Chili	4- 5	7
California Wonder	1- 6	2- 9
Chinese Giant	4	8
Large Bell or Bull Nose	7	10
Long Red Cayenne	4 7	10
Mexican Chili	7	8
Pimiento	3- 5	5-12
Red Chili	2- 4	10
Royal King	2- 3	3- 6
Ruby King	3- 6	5-12
Sweet Mountain	4- 6	10
Sweet Upright	4	5
Tobasco	2- 8	12-14
Ignacio pepper-tomato	3- 4	11
Novata pepper-tomato	3 - 5	11
Petaluma pepper-tomato	3- 5	12
San Geronimo pepper-tomato	6- 10	10
Sonoma pepper-tomato	3 - 4	8
Topepo pepper-tomato	2- 4	5- 6
Tulare pepper-tomato	3- 4	12

It is evident that the males lived from 1 to 10 days and the females from 2 to 14 days on the different varieties of peppers.

Tobacco (Nicotiana tabacum).—The following varieties of tobacco were experimentally infected with curly top: Big Havana, Connecticut Broad Leaf, Connecticut Seed Leaf, Sumatra, Turkish, and White Burley.

The first symptom to develop in the six varieties of tobacco experimentally infected with curly top was a clearing of the veinlets. A marked stunting of the infected plants occurred with a shortening of the internodes. The youngest leaves were dwarfed and outwardly cupped (fig. 5).
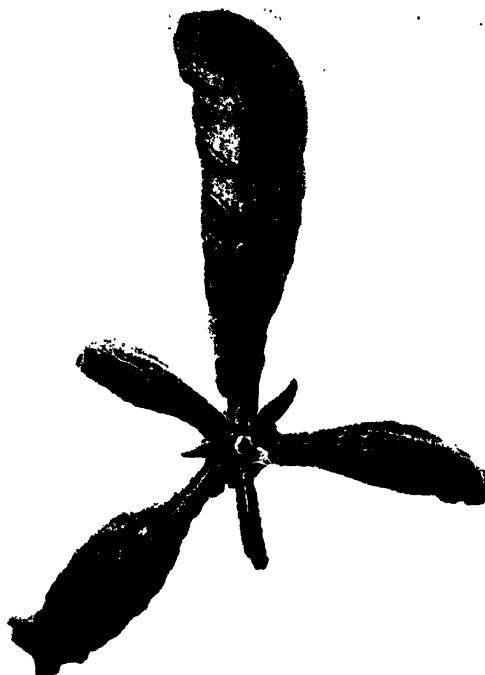

Fig. 5. White Burley tobacco (*Nicotiana tabacum*) experimentally infected with curly top, showing dwarfed youngest leaves, outward-cupped older leaves, and shortening of the internodes.

The adult life of the last living male and female beet leafhoppers on the different varieties of tobacco was as follows:

Variety of tobacco	Longevity of males *days*	Longevity of females *days*
Big Havana	4	5–14
Connecticut Broad Leaf	3– 4	6–12
Connecticut Seed Leaf	4– 8	4– 6
Sumatra	3	6–11
Turkish	4 10	3–10
White Burley	3– 5	13

The results show that the males lived from 3 to 10 days and the females from 3 to 14 days on a tobacco diet.

Fig. 6. Peasant's tobacco (*Nicotiana rustica*) experimentally infected with curly top, showing secondary shoots growing from the axil of the leaves, with dwarfed, inward-curled leaves.

Fig. 7. Peasant's tobacco (*Nicotiana rustica*): left, venation of healthy leaf; transparent venation on next two leaves from plant infected with curly top; right stem showing dwarfed youngest leaves with terminal ends rolled toward petioles.

Peasant's tobacco *(Nicotiana rustica)* was also experimentally infected with curly top. Secondary shoots frequently developed in the axil of the leaves (fig. 6). The younger leaves were dwarfed, inwardly curled (fig. 6) and sometimes the terminal ends of the youngest leaves rolled toward the petioles (fig. 7). The leaves showed the cleared veinlets. The average longevity of the adults on Peasant's tobacco varied according to the age of the plants, as follows:

Height of Peasant's tobacco	Longevity of males days	Longevity of females days
4–8 inches.	10	15
12–36 inches	59	63

Life History of Beet Leafhopper.—Nymphs which' hatched from eggs deposited in the following varieties of potatoes completed their life history: American Wonder, British Queen, Idaho Gems, Idaho Rurals, Red Prizetaker or Improved Early Rose, White Rose, White Rose Low-Top, and Wisconsin Pride. The beet leafhopper, however, failed to complete its life cycle on all varieties of peppers and tobaccos experimentally infected with curly top.

Weeds.—During the 1925 outbreak of the beet leafhopper, stunted plants of deadly nightshade *(Solanum nigrum douglassi)* with dwarfed leaves at the terminal end of the shoots, inward-curled older leaves, and secondary shoots growing from the axil of the leaves (fig. 8) were frequently observed in the beet fields of the Salinas Valley and in the San Joaquin Valley near Manteca. Three plants showing these symptoms were removed from the beet fields near Greenfield in the Salinas Valley and 12 were taken from the beet fields near Manteca. Non-infective beet leafhoppers after feeding on these 15 weeds transmitted curly top to 11 of 15 sugar beets. During the years between the 1919 and 1925 outbreaks of the beet leafhopper, this weed was rarely found to be naturally infected with the disease.

Deadly nightshade plants grown from seeds were repeatedly inoculated by different lots of from 5 to 50 infective male beet leafhoppers. The males lived from 3 to 11 days on this weed, and as soon as one batch of infective leafhoppers died another lot was placed on each plant. Non-infective hoppers after feeding on 10 plants inoculated with the disease by successive lots of 5 or 10 adults, transmitted curly top to 3 beets, while 7 beets remained healthy. One of 7 plants inoculated by successive lots of from 25 or 50 infective males, was shown to be experimentally infected with the disease. The experimentally infected plants showed a marked inward roll of the terminal leaves

(fig. 9). In all probability, mass infection is not a factor in curly-top infection of this weed. Whenever a severe epidemic of curly top occurs, the disease is apparently more virulent and weeds which are highly resistant seem to become more susceptible to the disease.

Fig. 8. Deadly nightshade (*Solanum nigrum douglassi*) naturally infected with curly top, showing secondary shoots growing from the axil of the leaves, with dwarfed leaves at the terminal end of the shoots.

Ground cherry *(Physalis wrightii)* growing in a beet field near Manteca was demonstrated to be naturally infected with curly top during the 1925 outbreak of the beet leafhopper.

Stramonium *(Datura stramonium)* was experimentally infected with curly top. The youngest leaves were dwarfed (pl. 2, fig. 2) and showed the transparent veinlets (pl. 2, fig. 2, insert).

Fig. 9. Deadly nightshade *(Solanum nigrum douglassi)*: left, shoot from check or control plant on which non-infective male beet leafhoppers had fed; right, five shoots and leaves from experimentally infected plants, showing inward-rolled youngest leaves.

CRUCIFERAE, MUSTARD FAMILY

During the 1925 outbreak of the beet leafhopper, numerous tests were made with a large number of Cruciferae to determine the natural host range of curly top in this family. Economic crucifers naturally infected with curly top are difficult to recognize in the field as most species show no reliable foliage symptoms of the disease. Plants on which the beet leafhoppers were collected in the field were removed with the root system, transplanted in flower pots and tested in the greenhouse.

Different varieties of crucifers grown from seeds were inoculated with curly top by infective beet leafhoppers, but the transmission of the disease by non-infective hoppers after feeding on the inoculated plants to sugar beets was not often accomplished. During the spring and summer of 1926, Henderson[4] inoculated a large number of crucifers, usually using 6 plants of each variety. In view of the fact that an occasional plant of some varieties was experimentally infected

with the disease, all varieties which were shown to be susceptible to curly top were again tested during 1927. During 1928, J. H. Freitag again repeated the work with all varieties which had previously been experimentally infected with the disease. During the four years a total of 18 plants of all susceptible varieties were inoculated by infective beet leafhoppers. The plants were usually inoculated after acquiring from 2 to 6 leaves.

Horse-Radish (Armoracia rusticana).—Horse-radish was demonstrated to be naturally infected with curly top. The beet leafhoppers were found abundant on June 17, 1925, in a quarter acre of horse-radish near Walnut Grove in the Sacramento Valley. The horse-radish was adjacent to a badly diseased beet field which had become so weedy that the beet foliage and weeds were cut by mowing. Nymphs in all stages of development were present on the foliage of horse-radish, and when the leaves were shaken the ground became covered with a multitude of hoppers. The horse-radish field was visited once a month from June to September, and an enormous increase of leafhoppers occurred, corresponding in numbers to those found in beet fields. In a vegetable garden on the Spreckels ranch near King City in the Salinas Valley the adults were scarce on a few horse-radish plants and no nymphs were observed.

Horse-radish plants with an inward curl of the leaves (fig. 10) were common. The sap exuded from the petioles of some of the leaves in a manner similar to curly-top beets. During the summer the foliage of many plants turned yellow. Horse-radish plants which had become dry were found here and there in this field during June, but in September many plants were dead.

The roots of horse-radish plants infected with curly top early in the season were dwarfed and brittle. A cross section of a diseased root shows darkened rings and bundles in the interior (pl. 3, fig. 2) while a longitudinal section shows the dark discolorations extending lengthwise through the root (pl. 3, figs. 1, 2).

Cuttings from diseased roots sometimes failed to sprout, or a large number of spindling shoots (pl. 3, fig. 1) developed and died before reaching or growing through the surface of the soil. If the shoots continued to grow the plant was stunted and the root did not increase in size.

Curly top was rarely transmitted from diseased horse-radish grown in this field to sugar beets. Twenty-two diseased horse-radish plants were removed from the soil with the root system and transplanted to flower-pots in the greenhouse. Non-infective beet leafhoppers after

feeding on the 22 plants transmitted curly top to 3 of 22 sugar beets. During September the youngest leaves and a few outer leaves with sap often exuding from the petioles were removed from a large number of plants. Thirty-six lots of non-infective males confined in cages were fed on the leaves for a period varying from 6 to 11 days, and were then transferred to 36 beet seedlings, but not a single case of curly top developed.

Fig. 10. Horse-radish (*Amoracia rusticana*), showing four inward-rolled leaves from a plant naturally infected with curly top.

It was decided to inoculate, with infective beet leafhoppers, some of the horse-radish plants grown in the field and others propagated from cuttings. Curly top was transmitted by non-infective males feeding on the inoculated plants to sugar beets from 22 of 29 horse-radish plants.

An experiment was now conducted to determine whether beet leafhoppers would continue to transmit curly top from the infected horse-radish plants during a period of three months. Eight lots of 25 non-infective males were fed on 8 infected horse-radish plants during each month and then each lot was equally distributed on 2 healthy beet seedlings. The results obtained are indicated in table 1.

TABLE 1

TRANSMISSION OF CURLY TOP FROM INFECTED HORSE-RADISH TO SUGAR BEETS
DURING A PERIOD OF THREE MONTHS

Horse-radish plant No.	Date of inoculation with infective males	Dates non-infective males fed on inoculated horse-radish plants					
		June 10-15		July 18-23		August 19-23	
		Beets infected	Beets healthy	Beets infected	Beets healthy	Beets infected	Beets healthy
1	May 21–June 10	1	1	2	0	0	2
2	May 21–June 10	1	1	0	2	1	1
3	May 21–June 10	1	1	2	0	0	2
4	May 21–June 10	2	0	2	0	1	1
5	May 11–June 10	2	0	1	1	0	2
6	May 10–June 10	2	0	0	2	0	2
7	May 10–June 10	1	1	0	2	1	1
8	May 21–June 10	2	0	0	2	0	2
Total......	. . ... .	12	4	7	9	3	13
Number of horse-radish plants from which disease was transmitted ...		8	......	4	. .	3	

Experiments were conducted to determine whether the virus becomes inactivated in horse-radish during the winter. Two of the 3 plants from which curly top was transmitted during a period of three months lived through the winter. During the following spring, non-infective beet leafhoppers after feeding on these 2 horse-radish plants failed to transmit curly top to beets.

Two shipments of naturally infected and apparently healthy roots were received from F. M. McKay during 1927. Cuttings were grown from 47 roots, some of which showed the dark discoloration, while others were white. Non-infective beet leafhoppers after feeding on the leaves of horse-radish were transferred to beets, but not a single case of curly top developed.

Another test was made to determine whether horse-radish plants in which the virus was inactivated during the winter could be reinfected with curly top. Eight plants with darkened areas in the roots were selected from the 47, and inoculated with curly top. Ten infec-

tive males were fed on each plant for a period of 25 days. Ten non-infective males were then fed from 3 to 6 days on each infected plant during each month for a period of four months. The monthly transmission of curly top to beets from such horse-radish plants as survived is shown as follows:

Month	Number of horse-radish plants alive	Number of beets that developed curly top	Number of beets healthy
First	8	8	0
Second	7	7	0
Third	6	5	1
Fourth	4	4	0

It appears that the resistant factors associated with the inactivation of the virus during the first year are greatly reduced or absent when horse-radish is reinfected during the second year.

Observations and experiments conducted in the field and greenhouse by McKay indicate that horse-radish from late-infected plants may be white, but when white cuttings were planted, some of these were badly discolored when dug and resulted in a diseased growth in a high percentage of cases. It is evident that after a serious outbreak of curly top in horse-radish fields, cuttings should be made from healthy roots obtained from the Middle West or East, where curly top does not occur.

Radish (Raphanus sativus).—A variety of radish, probably Red Globe, growing in a vegetable garden of the Spreckels ranch near Greenfield in the Salinas Valley, was naturally infected with curly top. The disease, however, was transmitted to sugar beets from only 2 of 15 plants.

Long Black Spanish and Long White Japanese radishes were experimentally infected with curly top, but 17 varieties were not infected with the disease.

The naturally infected radishes showed an inward curl of the leaves with outstanding veins (fig. 11). The two varieties of radishes experimentally infected with curly top showed no reliable foliage symptoms under greenhouse conditions, except a stunting of the plants and a yellowing of the leaves in the later stages of the disease. Long Black Spanish showed a slight inward roll of some of the leaflets, but this also occurred in radishes used as a check or control.

Collards (Brassica oleracea acephala).—Georgia Southern or Creole and True Southern collards were experimentally infected with curly top.

One plant of Georgia Southern or Creole collards showed a dwarfing and malformation of the youngest leaves with shortened petioles (fig. 12) but other infected plants developed no such symptoms.

Fig. 11. Radish (*Raphanus sativus*) naturally infected with curly top, showing inward-curled leaves with outstanding veins. The variety was determined by J. T. Rosa as probably Red Globe.

Garden Cabbage (Brassica oleracea capitata).—Curly top was transmitted to sugar beets from an unknown variety of cabbage growing in a vegetable garden of the Spreckels Sugar Company near King City in the Salinas Valley during 1925.

The following varieties of cabbage were experimentally infected with curly top: All Head Early, Early Jersey Wakefield, Early Winningstadt, Improved American Savoy, Charleston, Wakefield, Large Flat Dutch, Large Late Drumhead, Mammoth Red Rock, and Surehead.

Among all of the experimentally infected varieties of cabbages listed, infective beet leafhoppers inoculated many plants from which

Fig. 12. Georgia Southern or Creole collards (*Brassica oleracea acephala*) experimentally infected with curly top, showing dwarfed and malformed youngest leaves with shortened petioles.

non-infective males failed to transmit the disease to sugar beets. In some of the experiments different varieties of cabbages were planted on the same date, infected with the same number of hoppers, and exposed to the same temperatures and sunlight; but under these conditions some plants were infected with curly top while others were not.

Naturally infected cabbage showed no reliable foliage symptoms of curly top. Sap sometimes exuded from the petioles and veins of varieties infected in the greenhouse.

Cauliflower, Broccoli (Brassica oleracea botrytis).—Early Snowball, Large White Cape, Purple Cape, and St. Valentine were experi-

mentally infected with curly top. Two St. Valentine broccoli showed a whitening of the veinlets on a portion of the younger leaves, but non-infective beet leafhoppers failed to transmit curly top to sugar beets from these plants. The white venation disappeared later.

Turnip (Brassica rapa).—Curly top was transmitted to sugar beets from 7 to 35 stunted Purple Top Globe turnips growing in a vegetable garden of the Spreckels ranch near Greenfield.

The following varieties of turnips were experimentally infected with curly top: Cow Horn or Long White, Early Purple Top, Flat Dutch, and Seven Top.

Fig. 13. Terminal shoot of White London mustard (*Brassica alba*) experimentally infected with curly top, showing youngest leaves curled inward along the mid-rib.

Turnips showed no reliable foliage symptoms of curly top. In some varieties a cupping or slight inward rolling of the leaves developed, but this also occurred in the check or control plants. One plant of Cow Horn or Long White turnip showed a shortening of the petioles of the youngest leaves, but other infected plants were apparently normal.

White London Mustard (Brassica alba).—White London mustard was experimentally infected with curly top. The youngest leaves of infected plants were dwarfed and curled inward along the mid-rib (fig. 13).

Chinese, Celery Cabbage, or Wong Bok (Brassica pekinensis).— Four plants removed from the field and 8 plants grown from seeds were non-susceptible to curly top.

Chinese Cabbage, probably Chosen and Pe-tsai (Brassica pekinensis).—Two varieties of Chinese cabbage appeared to be naturally infected with curly top; the youngest leaves were dwarfed and curled, but non-infective beet leafhoppers failed to transmit the disease to sugar beets.

Cress (Barbarea vulgaris).—Two varieties of cress, Fine Curled and True Water, were experimentally infected with curly top.

The youngest leaves of Fine Curled cress were curled (fig. 14) and showed faint indications of transparent venation. True Water

Fig. 14. Fine Curled cress (*Barbarea vulgaris*): four curled leaves from a plant experimentally infected with curly top; right, leaf from check or control plant on which non-infective male beet leafhoppers fed.

cress showed (pl. 2, fig. 1) a shortening of the petioles at the terminal end of the shoots, an inward roll of the leaflets, and sometimes elevations on the lower surface of the leaves.

Natural Breeding Plants of Beet Leafhopper.—Nymphs which hatched from eggs deposited in the foliage of the following Cruciferae removed from the field acquired the winged stage: horse-radish, unknown variety of cabbage, and Purple Top Globe and Early White Flat Dutch turnips. Nymphs in all stages of development and adults were abundant on horse-radish, and Purple Top Globe and Early White Flat Dutch turnips, but rare on garden cabbage during 1925. Adults were commonly found on radish but no nymphs were observed when the foliage was disturbed with the hand.

Life History of Beet Leafhopper.—The beet leafhopper completed its life history in the greenhouse on the following varieties of Cruciferae:

Horse-radish (*Armoracia rusticana*).

Radish (*Raphanus sativus*): California or Chinese White Winter, China Rose Winter, Crimson Giant, Early Scarlet Globe, Early Scarlet Turnip, French Breakfast, Half Long Deep Scarlet, Icicle or White Icicle, Long Black Spanish, Long Scarlet, Long White Japanese, Round Black Spanish, Siberian, Winter or Chinese White Winter, White Tip Scarlet Turnip, and White Vienna.

Kale, borecole (*Brassica oleracea acephala*): Dwarfed Curled. Scotch, Giant Marrow, Jersey or Thousand Headed, and Tall Curled Scotch.

Collards (*Brassica oleracea acephala*): True Southern.

Brussels sprouts (*Brassica oleracea gemmifera*): Cooper's Selected Aigbruth, Danish Giant, Dwarf Perfection, and Morse's Brussels sprouts.

Garden cabbage (*Brassica oleracea capitata*): All Head Early, All Season, Autumn King, Copenhagen Market, Danish Ball Head, Drumhead Savoy, Early Flat Dutch, Early Jersey Wakefield, Early Winningstadt, Improved American Savoy, Late Flat Dutch, Large Early Wakefield, Large Flat Dutch, Large Late Drumhead, Mammoth Red Rock, and Surehead.

Cauliflower, Broccoli (*Brassica oleracea botrytis*): California Wonder, Dry Weather, Early Snowball, Improved Autumn Giant, Large White Cape, Morse's February, Morse's April, Purple Cape, St. Valentine, and Veitch's Autumn Giant.

Kohlrabi (*Brassica oleracea caulorapa*): Early White Vienna and Early Purple Vienna.

Rutabaga (*Brassica napobrassica*): American Purple Top and Purple Top Yellow.

Turnip (*Brassica rapa*): Amber Globe, American Purple Top, Cow Horn or Long White, Early Purple Top, Early Purple Top Milan, Early White Flat Dutch, Orange Jelly or Golden Ball, Purple Top Strapped-leaved, Purple Top White Globe, Seven Top, Snowball, White Egg, and White German.

Chinese mustard (*Brassica juncea*).

Southern Curled mustard (*Brassica juncea crispifolia*).

Chinese, Celery cabbage, or Wong Bok (*Brassica pekinensis*).

Black mustard (*Brassica nigra*).

White London mustard (*Brassica alba*).

Weeds.—Charlock *(Brassica arvensis)* growing in a beet field near Hamilton City in the Sacramento Valley was demonstrated to be naturally infected with curly top during 1918.

Shepherd's purse *(Capsella bursa-pastoris)* growing in a beet field near Greenfield in the Salinas Valley was proved to be naturally infected with curly top during 1925. This weed grown from seed was also experimentally infected with the disease. The infected plants were stunted with twisted seed stalks (fig. 15) usually bearing malformed seeds (fig. 16) near the terminal ends.

Fig. 15. Shepherd's purse (*Capsella bursa-pastoris*) naturally infected
with curly top, showing twisted seed stalks.

Fig. 16. Shepherd's purse (*Capsella bursa-pastoris*): upper row, terminal ends
of twisted seed stalks, showing malformed seeds; lower row, seeds from lower
region of seed stalks.

POLYGONACEAE, BUCKWHEAT OR KNOTWEED FAMILY

Common Buckwheat (Fagopyrum esculentum).—Common buck-wheat was experimentally infected with curly top. The first symptoms to appear were blister-like elevations on the youngest leaves, then the margins of the youngest leaves rolled inward (fig. 17), with the tissue sunken between the lateral veins, and twisted petioles (fig. 17). In the later stages of the disease the leaves were dwarfed and often cupped outward. The plant finally turned yellow and died.

Fig. 17. Common buckwheat (*Fagopyrum esculentum*): left, tip of plant experimentally infected with curly top, showing inward-rolled youngest leaves with twisted petioles and curled older leaves; right, check or control plant on which non-infective beet leafhoppers fed.

Giant Crimson Winter Rhubarb (Rheum rhaponticum).—Giant Crimson Winter rhubarb was experimentally infected with curly top, but showed no reliable foliage symptoms. Curly top was transmitted to sugar beets from 7 of 13 plants inoculated by infective beet leafhoppers.

French Large-leaved Sorrel (Rumex scutatus).—French Large-leaved sorrel experimentally infected with curly top developed wart-

like protuberances (fig. 18) on the lower surface of the leaves in the later stages of the disease.

Life History of Beet Leafhopper.—Nymphs which hatched from eggs deposited in the following plants of the Polygonaceae completed their life history in the greenhouse: Common buckwheat, French Large-leaved sorrel, Giant Crimson Winter, and Wagner's Giant rhubarb.
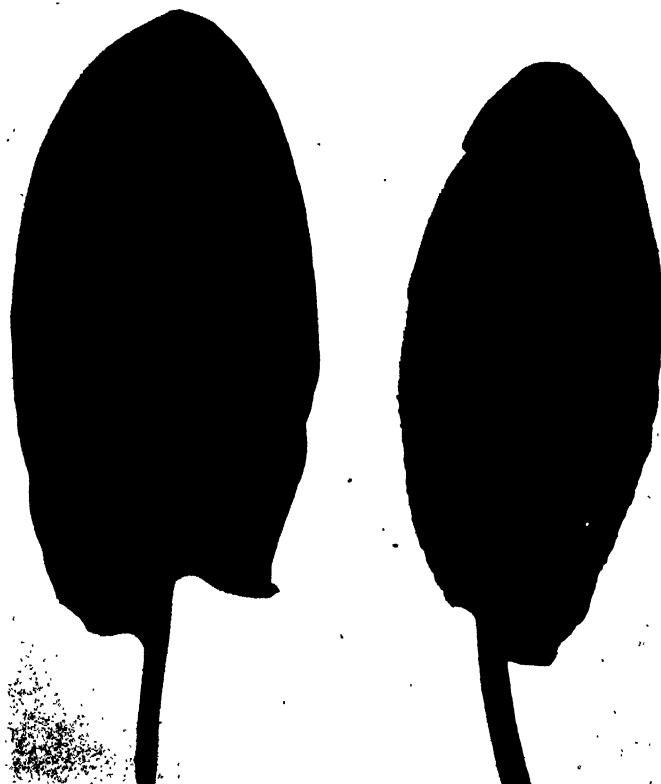

Fig. 18. French Large-leaved sorrel (*Rumex scutatus*): left, leaf from check or control plant on which non-infective beet leafhoppers fed; right, leaf from plant experimentally infected with curly top, showing wart-like protuberances on lower surface.

Weeds.—The following weeds were proved to be naturally infected with curly top: wire grass *(Polygonium ariculare)*, swamp smartweed *(P. muhlenbergii)* (fig. 19), water smartweed *(P. amphibian hartwrightii)*, common knotweed *(P. lapathifolium)*, and a lady's thumb *(P. persicaria)*.

Curly dock *(Rumex crispus)* was experimentally infected with curly top and showed wart-like protuberances (fig. 20) on the lower surface of the leaves in the later stages of the disease. The protuberances of the lower surface of the leaves is a reliable and constant symptom of curly top in most economic plants of the Chenopodiaceae, but this symptom failed to develop in cultivated plants and weeds of all families so far investigated except sorrel *(Rumex sculatus)* and curly dock *(R. crispus)* of the family Polygonaceae.

Fig. 19. Swamp smartweeds *(Polygonium muhlenbergii)* naturally infected with curly top, showing inward-rolled leaves.

Fig. 20. Curly dock (*Rumex crispus*): left, leaf from a plant experimentally infected with curly top, showing wart-like protuberances on the lower surface; right, leaf from check or control plant on which non-infective beet leafhoppers fed.

UMBELLIFERAE, PARSLEY FAMILY

Coriander (Coriandrum sativum).—Coriander experimentally infected with curly top showed a marked curling and twisting of the leaflets (fig. 21).

Dill (Anethum graveolens).—Dill was inoculated with curly top by infective beet leafhoppers in the greenhouse and showed a drooping of the leaflets from the curved mid-rib (fig. 22).

Florence Fennel (Foeniculum dulce).—Florence fennel experimentally infected with curly top showed a shortening of the petioles

Fig. 21. Coriander (*Coriandrum sativum*): left, leaf from a plant experimentally infected with curly top, showing curled and twisted leaflets; right, leaf from check or control plant.

Fig. 22. Dill (*Anethem graveolens*): left, two leaves from a plant experimentally infected with curly top, showing drooping of leaflets from curved midrib; right, leaf from check or control plant on which non-infective beet leafhoppers fed.

of the youngest leaves, with curled thread-like leaflets (fig. 23). The petioles of the somewhat older leaves often drooped (fig. 23).

Parsley (Petroselinum hortense).—Plain parsley was proved to be naturally infected with curly top in the Salinas and Santa Clara valleys. During the 1925 outbreak of the beet leafhopper, nymphs

Fig. 23. Florence fennel (*Foeniculum dulce*) experimentally infected with curly top, showing shortened petioles of youngest leaves and curled thread-like leaflets.

and adults were commonly found on parsley growing in the vegetable garden of Spreckels Sugar Company near Chular. Curly top was transmitted to sugar beets from only 2 of 16 plants removed from the field.

The following varieties of parsley were experimentally infected with curly top: Champion Moss-Curled, Fern-Leaf Moss, and Plain.

The disease, however, was rarely transmitted to sugar beets from the different varieties of parsley inoculated by infective beet leafhoppers.

Naturally and experimentally infected parsley showed no visible foliage symptom of the disease.

Celery (Apium graveolens dulce).—The results of the investigations on celery infected with curly top in the greenhouse has been published in a previous paper.[8]

Salad Chervil (Anthriscus cerefolium).—Salad chervil was infected with curly top in the greenhouse. The symptoms were similar to those of Florence fennel.

Life History of Beet Leafhopper.—Nymphs which hatched from eggs deposited in the following varieties of Umbelliferae acquired the adult stage on these food plants: caraway (*Carum carvi*), coriander, Florence fennel; Champion Moss-Curled, Fern Leaf-Moss, Hamburg or Turnip-Rooted and Plain parsley; Giant Paschal, Golden Self-Blanching and White Plume celery; and salad chervil.

MALVACEAE, MALLOW FAMILY

Okra or Gumbo (Hibiscus esculentus).—The following varieties of okra or gumbo were experimentally infected with curly top: Long Green, Perkins Mammoth Long Pod, and White Velvet.

The three infected varieties of okra or gumbo were stunted and showed a slight outward cupping of the youngest leaves. The lower leaves turned yellow and dropped from the plants.

The longevity of the last living male and female beet leafhopper on the three varieties of okra or gumbo was as follows:

Variety of okra or gumbo	Longevity of males *days*	Longevity of females *days*
Long Green	3–12	3–15
Perkin's Mammoth Long Pod ...	3–13	8–20
White Velvet	3–16	14–19

Nymphs which hatched from eggs deposited in okra or gumbo failed to complete their life cycle in the greenhouse.

Acala Cotton (Gossypium hirsutum).—A number of letters were received inquiring whether Acala cotton is naturally infected with curly top. An examination of many fields of cotton in the San Joa-

quin Valley failed to show any indication of this disease nor of the beet leafhopper. These observations were made in years between outbreaks of the beet leafhopper; no examinations were made during the 1925 outbreak of the pest.

Acala cotton is non-susceptible to curly top. Twenty plants grown from seeds were repeatedly inoculated by different lots of 10 infective beet leafhoppers. As soon as the 10 hoppers had died on each plant another batch of 10 was put in the cage enclosing each plant. In this experiment 1,000 infective males were used to inoculate the 20 plants.

Fig. 24. Dwarf mallow (*Malva rotundifolia*): left, plant experimentally infected with curly top, showing drooping youngest leaves; right, check or control plant on which non-infective male beet leafhoppers fed.

Twenty lots of 15 non-infective males after feeding on each inoculated cotton plant were distributed on 40 beets, but not a single case of curly top developed.

The adult life on Acala cotton was as follows: males, 1–11 days; females, 4–12 days.

Weeds.—Dwarf mallow *(Malva rotundifolia)* and cheeseweed *(M. parviflora)* were proved to be naturally infected with curly top. During the 1919 and 1925 outbreaks of the beet leafhopper dwarfed mallow was demonstrated to be naturally infected with the disease in the fog belt and interior regions of the Salinas Valley. Diseased dwarfed mallow was common in the beet and bean fields and along roadsides during 1925 in the Salinas Valley. Cheeseweed showing symptoms of curly top was commonly found in the beet fields of the

San Joaquin and Sacramento valleys during 1919 and in later years. *M. rotundifolia* (fig. 24) and *M. parviflora* grown from seeds were also experimentally infected with the disease in the greenhouse.

Nymphs which hatched from eggs deposited in the leaves of *Malva rotundifolia* and *M. parviflora* under natural conditions completed their life cycle on these food plants in the greenhouse.

LINACEAE, FLAX FAMILY

Flax (Linum usitatissimum).—Flax experimentally infected with curly top was stunted, with the leaves clustered close together and twisted (pl. 4, fig. 1) at the terminal end of the shoots. The longitudinal veins were wavy with blister-like elevations (pl. 4, fig. 2). In a later stage of the disease the plant turned yellow and died. The twisting of the leaves began four days after infection.

Nymphs which hatched from eggs deposited in flax completed their life cycle.

BORAGINACEAE, BORAGE FAMILY

Borage or Bee-Plant (Borago officinalis).—Borage or bee-plant was experimentally infected with curly top in the greenhouse. The youngest leaves were curled inward with outstanding lateral veins. In the later stages of the disease, the leaves turned yellow and the plant died. Small plants infected with curly top succumbed rapidly from the effects of the disease.

The life cycle was completed by nymphs which hatched from eggs deposited in this plant.

VALERIANACEAE, VALERIAN FAMILY

Corn Salad (Valerianella locusta olitoria).—Corn salad was experimentally infected with curly top. The youngest or innermost leaves of infected plants were curled and dwarfed (fig. 25). In the later stages of the disease, the plant turned yellow and died.

Nymphs which hatched from eggs deposited in corn salad completed their life history on this food plant in the greenhouse.

Fig. 25. Corn salad (*Valerianella locusta olitoria*): left, plant experimentally infected with curly top, showing curled, dwarfed youngest leaves; right, check or control plant on which non-infective beet leafhoppers fed.

ATTENUATED VIRUS

Carsner[1] lists the following plants under their scientific names as non-susceptible to curly top. The common names have been added.

Chenopodiaceae—
   Chenopodium leptophyllum.
   Chenopodium murale (nettle-leaf goosefoot).

Leguminosae—
   Phaseolus vulgaris (pink bean).

Cucurbitaceae—
   Cucumis melo (muskmelon).
   Cucumis sativus (cucumber).

Solanceae—
   Solanum nigrum (deadly nightshade).
   Capsicum sp. (pepper).

Cruciferae—
   Brassica oleraceae (?).
   Raphanus sativus (radish).

Polygonaceae—
   Rumex crispus (curly dock).

Umbelliferae—
   Apium graveolens (celery).

It is evident from the results of the investigation given in this and in a previous paper[9] that all of the above economic plants and weeds were experimentally infected with curly top in the greenhouse and many were also proved to be naturally infected with the disease in the field. An examination of the list of cultivated plants also shows that the plants of all families with the exception of the Cucurbitaceae often required a large number of tests before a plant was experimentally infected with curly top.

In a later paper, Carsner[2] reports that the virus of curly top becomes so attenuated when passed through certain weeds such as *Chenopodium murale, Rumex crispus,* and *Suaeda moquini* that it causes merely a mild form of curly top when transferred to healthy beets or other susceptible plants. The attenuated virus was trans-mitted from 13 of 33 plants of *C. murale* inoculated by infective beet leafhoppers, but non-infective hoppers failed to transmit the disease from 20 plants.

No experiments have been performed up to the present time to prove that the virus is attenuated in resistant cultivated plants which were naturally or experimentally infected with curly top.

SUMMARY

The following varieties of economic plants have been found to be naturally infected with curly top in California:

Solanaceae, nightshade family—
> Potato (*Solanum tuberosum*): unknown variety.
> Tomatoes (*Lycopersicon esculentum*): all varieties grown in California.[7]
> Peppers: Anaheim Chili, Paprika, Pimiento, and Mexican Chili (*Capsicum frutescens*).

Cruciferae, mustard family—
> Horse-radish (*Armoracia rusticana*).
> Radish (*Raphanus sativas*): variety doubtful, probably Red Globe.
> Cabbage (*Brassica oleracea capitata*): unknown variety.
> Turnip (*Brassica rapa*): Purple Top Globe.

Umbelliferae, parsley family—
> Parsley (*Petroselinum hortense*): Plain.

The following varieties of cultivated plants were experimentally infected with sugar-beet curly top:

Solanaceae, nightshade family—
> Potatoes (*Solanum tuberosum*): American Wonder, British Queen, Idaho Gems, Idaho Rurals, Red Prizetaker or Improved Early Rose, White Rose, White Rose Low-Top, and Wisconsin Pride.

Tomatoes (*Lycopersicon esculentum*): Alameda Trophy, Earliana, First Early, Globe, King of the Earlies, San Jose Canner, Santa Clara Canner, Special Early, Stone, and Wild Mexican.[7]

Peppers (*Capsicum frutescens*): Anaheim Chili, California Wonder, Chinese Giant, Large Bell or Bull Nose, Long Red Cayenne, Mexican Chili, Pimiento, Red Chili, Royal King, Ruby King, Sweet Mountain, Sweet Upright, and Tobasco.

Pepper-tomatoes: Ignacio, Novata, Petaluma, San Geronimo, Sonoma, Topepo, and Tulare.

Tobacco (*Nicotiana tabacum*): Big Havana, Connecticut Broad Leaf, Connecticut Seed Leaf, Sumatra, Turkish, and White Burley; Peasants' tobacco (*N. rustica*).

Cruciferae, mustard family—

Horse-radish (*Armoracia rusticana*).

Radish (*Raphanus sativus*): Long Black Spanish and Long White Japanese.

Collards (*Brassica oleracea acephala*): Georgia Southern, or Creole, and True Southern.

Garden cabbage (*Brassica oleracea capitata*): All Head Early, Early Jersey Wakefield, Early Winningstadt, Improved American Savoy, Charleston Wakefield, Large Flat Dutch, Large Late Drumhead, Mammoth Red Rock, and Surehead.

Cauliflower, or broccoli (*Brassica oleracea botrytis*): Early Snowball, Large White Cape, Purple Cape, and St. Valentine.

Turnip (*Brassica rapa*): Cow Horn or Long White, Early Purple Top, Flat Dutch, and Seven Top.

Mustard (*Brassica alba*): White London mustard.

Cress (*Barbarea vulgaris*): Fine Curled (pepper grass) and True Water.

Polygonaceae, buckwheat or knotweed family—

Buckwheat (*Fagopyrum esculentum*): Common Rhubarb (*Rheum rhaponticum*) Giant Crimson Winter.

Sorrel (*Rumex scutatus*): French Large-leaved.

Umbelliferae, parsley family—

Coriander (*Coriandrum sativum*).

Dill (*Anethum graveolens*).

Florence fennel (*Foeniculum dulce*).

Parsley (*Petroselinum hortense*): Champion Moss-Curled, Fern-Leaf Moss, and Plain.

Celery (*Apium graveolens dulce*): Giant Paschal, Golden Self-Blanching and White Plume.[8]

Salad chervil (*Anthriscus cerefolium*).

Malvaceae, mallow family—

Okra or gumbo (*Hibiscus esculentus*).

Linaceae, flax family—

Flax (*Linum usitatissimum*).

Boraginaceae, borage family—

Borage or bee-plant (*Borago officinalis*).

Valerianaceae, valerian family—

Corn salad (*Valerianella locusta olitoria*).

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PLATE 1

Pepper-Tomato

Right, leaf from Novata pepper-tomato used as a check or control on which ten non-infective male beet leafhoppers fed. The other six terminal shoots or leaves are from six varieties of pepper-tomato plants each experimentally infected with curly top by 10 infective males. Upper row, right to left: Novata, Ignacio, and Petaluma pepper-tomatoes; lower row, right to left: Tulare, San Geronimo, and Sonoma pepper-tomatoes.

PLATE 2

True Water Cress (*Barbarea vulgaris*)

Fig. 1. Terminal shoots and leaves of True Water cress experimentally infected with curly top. Terminal shoots show shortened petioles of youngest leaves. Leaves show inward roll of leaflets. The right-hand leaf in the upper row shows blister-like elevations on the lower surface of terminal leaflets.

STRAMONIUM (*Datura stramonium*)

Fig. 2. Terminal end of plant experimentally infected with curly top, showing dwarfed youngest leaves. Insert, leaf showing transparent venation.

Fig. 1

Fig. 2

Horse-Radish (*Amoracia rusticana*)

Fig. 1. Horse-radish plants grown from different root cuttings. The two at the right were white when planted and are assumed to be healthy. The three in the center were white when planted, but discolored when dug, and show the spindly growth from a large number of eyes scattered at different places over the roots. The three at the right were discolored when planted and gave also very poor growth from many eyes. Courtesy M. B. McKay.

Fig. 2. Cross and longitudinal sections of horse-radish roots infected with curly top, showing dark discolorations. Courtesy M. B. McKay.
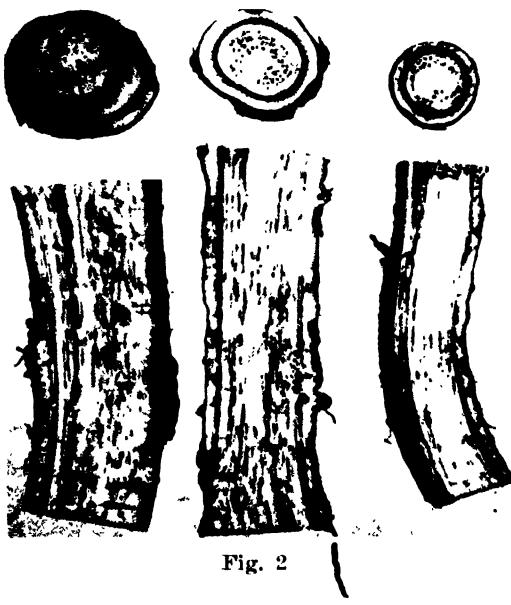
Fig. 1

Fig. 2

PLATE 4

Flax (*Linum usitatissimum*)

Fig. 1. Left, shoot of check or control plant on which non-infective beet leafhoppers fed; right, tip of a plant experimentally infected with curly top, showing leaves clustered close together and twisted at the terminal end of the shoot.

Fig. 2. Leaves from an infected plant, showing wavy longitudinal veins with blister-like elevations.

Fig. 1

Fig. 2